

MANAGEMENT OF BACTERIAL BLIGHT
(*Xanthomonas axonopodis* pv. *malvacearum*) IN RAINFED
Bt COTTON”

By
KHARAT K.Y.
B.Sc. (AGRI.)

MASTER OF SCIENCE
(Agriculture)
IN
PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, PARBHANI
VASANTRAO NAIK MARATHWADA KRISHI VIDYAPEETH,
PARBHANI, 431 402 (M.S.), INDIA

2018

VISIT...

LANZAROTE
Caliente.COM

MANAGEMENT OF BACTERIAL BLIGHT
(*Xanthomonas axonopodis* pv. *malvacearum*) IN RAINFED
Bt COTTON”

By
KHARAT K.Y.
B.Sc. (AGRI.)

DISSERTATION
Submitted to
Vasantrao Naik Marathwada Agricultural University,
Parbhani
in partial fulfilment of the requirements
for the Degree of

MASTER OF SCIENCE
(Agriculture)
IN
PLANT PATHOLOGY

DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE, PARBHANI
VASANTRAO NAIK MARATHWADA KRISHI VIDYAPEETH,
PARBHANI, 431 402 (M.S.), INDIA

2018

CANDIDATE DECLARATION

*I hereby declare that the dissertation
or part thereof, has not been
previously submitted by
me for a degree of
any University.*

Place : Parbhani

(KHARAT K.Y.)

Date : / /

Reg.No.2016A/95M

Dr. P.K. Dhoke

Assistant Prof. (Plant Pathology)

Cotton Research Station, Nanded

VNMKV, Parbhani

CERTIFICATE – I

This is to certify that the dissertation entitled **MANAGEMENT OF BACTERIAL BLIGHT (*Xanthomonas axonopodis* pv. *malvacearum*) IN RAINFED Bt COTTON**” submitted by **MR. KHARAT K.Y. (Reg. No. 95M/2016A)** to the Vasant Rao Naik Marathwada Krishi Vidhyapeeth, Parbhani in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** is record of original and bonafide research work carried out by her under my guidance and supervision. It is of sufficiently high standard to warrant its presentation for the award of the said degree.

I also certify that the dissertation or part thereof has not been previously submitted by her for a degree of any university.

Place: Parbhani

Date :

(Dr.P.K.DHOKE)

Research Guide

CERTIFICATE – II

This is to certify that the dissertation entitled **MANAGEMENT OF BACTERIAL BLIGHT (*Xanthomonas axonopodis* pv. *malvacearum*) IN RAINFED Bt COTTON**

submitted by **MR. KHARAT K.Y. (Reg. No. 95M/2016A)** to the Vasantao Naik Marathwada Krishi Vidhyapeeth, Parbhani in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (Agriculture)** in the subject of **PLANT PATHOLOGY** has been approved by the student's advisory committee after *viva-voce* examination in collaboration with the external examiner.

(
External Examiner

(Dr.P.K.DHOKE)
Research Guide

Members of Advisory Committee:

(Dr. V.G. Mulekar)

(Dr. C.V. Ambadkar)

(Dr. D.G. More)

Associate Dean (P.G.)
College of Agriculture,
VNMKV, Parbhani

ACKNOWLEDGEMENT

I am obligated to pay thanks in the “lotus feet” of the “Almighty”, omnipresent God for having been my guiding light, driving force for giving me strength, courage and fortitude to achieve this goal and endurance to go through all important crossroads.

*I take this golden opportunity to express my heartiest and deepest sense of gratitude to those who helped me to complete my research work. The feelings of gratitude, when expressed in words, is only its very small acknowledgement and pay my regard to my revered and learned teacher **Dr. P.K.Dhoke**, Assistant Professor, Cotton research station Nanded. This work could not have been undertaken, far less completed, without his scholarly guidance for being suggested the research problem in initial stage, invaluable suggestions, critical supervision, a constant source of refreshing encouragement, and inspiration to me. But that this work has been compiled is a mash of the great pains he took and the precious time he spared in facilitating the things for me whenever I faltered during execution /completion of this research work.*

*I offer my sincere thanks to him and seek his blessings to be even an iota of what he has been. I will be failing in my moral duty if would not mention the name of my all times respected Head, **Dr. D.N. Dhutraaj**, Department of Plant Pathology for his encouragement during my study period. It is with extreme pleasure that I thank to **Dr. K.T. Apet** Associate professor, Dept. of Plant Pathology, **Dr. K.S. Baig**, cotton specialist, Cotton Research Station, Nanded **prof. A.D Pandagale**, Farm supridendant Cotton Research Station, Nanded, **Dr. K. D. Navgire**, Associate professor, Dept. of Plant Pathology, **S.N. Badgujar**, Associate professor, Dept. of Plant Pathology, **Dr. C.V. Ambadkar**, Assistant professor, Dept. of Plant Pathology, **Shri. P.L.***

Sontakke, Assistant Professor, department of Plant Pathology, members of my advisory committee for their kind help and various courtesies extended during my research work. I wish to record my sincere thanks to **Dr. B. Venkateshwaralu** Hon'ble Vice-Chancellor, **Dr. V. D. Patil**, Dean College of Agriculture, Parbhani, **Dr. D.N. Gokhale**, Associate Dean and Principal, College of Agriculture, VNМКV, Parbhani. The true pleasure while working in the department was due to the technical staff as they made things available on time and rendered their help whenever required. My specially thanks to **Laxmam mama**, **Kadam mama**, **Manikrao**, **Yakub mama**, **Chapate mama** for the timely help, affectionate encouragement and useful suggestions during the tenure of this investigation.

One needs sincere friend at all major juncture in life to bear strains and fatigue chaeerfully. I have been very lucky in this respect. I would like to thanks **Ms. Reshma** to her contribution towards my thesis work. He helped me and boosted my moral and steered my thesis work in various ups and down.

Words run short to express the feelings towards my colleagues **Nikhil**, **Swati**, **Kiran**, **Neha**, **Pooja** , **Shital**, **Bhushan** and **Buddhisagar** **Yogesh**, **Rajnikant**, **Samadhan**. The moral support and ever ready helping nature of my all classmates. My loving juniors **swapnil** for their full cooperation during the research programme.

In the last but not the least words are too less to express my sincere gratitude to my beloved parents Words are inadequate to express my illimitable veneration, adoration, indebtness and gratitude towards my reverend parents, whose constant inspiration, blessing, everlasting love and innumerable sacrifice have encouraged me in every step of my life, not only to go through gigantic task but to have pride to succeed in every walk of life. Emotions of my heart should

find new boundaries to contain my love for my loving mother & father for their love and affectionable motivation to me every time, whenever I needed.

Lastly, I express my regards to those whose names I have forgotten to mention here.

Place : Parbhani

(Kharat.K.Y)

Date :

CONTENTS

Chapter	Title	Page No.
1.	INTRODUCTION	1-4
2.	REVIEW OF LITERATURE	5-32
3.	MATERIALS AND METHODS	33-42
4.	RESULTS AND DISCUSSION	43-61
5.	SUMMARY AND CONCLUSIONS	62-65
	LITERATURE CITED	i – xiii
	THESIS ABSTRACT	
	APPENDIX	
	VITAE	

LIST OF TABLES

TABLE NO.	CONTENTS	PAGE NO.
1.	Survey of Bacterial blight of cotton in Nanded and Parbhani districts.	43
2.	Morphological characteristics of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	46
3.	Cultural characteristics of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> on different agar media	48
4.	Cultural characteristics of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> on different agar slants	48
5.	Cultural characteristics of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> in different broth	49
6.	<i>In vitro</i> efficacy of chemicals agent against <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	49
7.	<i>In vivo</i> efficacy of chemicals agent on per cent disease incidence of <i>Xam</i> of Bt cotton	51
8.	<i>In vivo</i> efficacy of chemicals agent on disease severity of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> of Bt cotton	54
9.	study the host range of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	57
10.	Economics of antibiotics and fungicides sprayings for the management of bacterial blight of cotton Cv. Jadoo BGII during <i>Kharif</i> , 2017	59
11.	Effect of inoculation of <i>Xanthomonas axonopodis</i> Pv. <i>malvacearum</i> on field crops.	61
12.	Effect of inoculation of <i>Xanthomonas axonopodis</i> Pv. <i>malvacearum</i> plantation crops and tree.	61

LIST OF PLATES

PLATE NO.	CONTENTS	In between pages
I (A,B)	Survey of Bacterial blight of cotton	43-44
II (A,B,C)	Typical symptoms of <i>Xam</i> on Bt cotton (var. Jaadoo BG-II)	46-49
III	Typical colonies of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	47-48
VI (A,B)	Morphological characteristics of <i>Xanthomonas</i> <i>axonopodis</i> pv. <i>malvacearum</i>	48-49
V (A)	Cultural characteristics of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> on different agar media	48-49
V (B,C)	Cultural characteristics of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i> on different agar plates and broth	49-50
VI (A,B)	Pathogenicity of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	51-52
VII	<i>In vitro</i> efficacy of chemicals at different concentrations against <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	51-52
VIII (A,B,C)	General view of Bt cotton field	54-55

LIST OF FIGURES

Fig. No.	CONTENTS	In between pages
1.	Field layout of research on bacterial blight of Bt cotton (var. Jaadoo BG-II)	39-40
2	Map of Marathwada Region showing district wise average bacterial blight incidence	43-44
2	Survey of Bacterial blight of cotton in Nanded and Parbhani districts.	45-46
4.	<i>In vitro</i> efficacy of chemicals against <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>	51-52
5.	<i>In vivo</i> efficacy of chemicals per cent disease incidence and severity of <i>Xam</i> of Bt cotton	54-55

ABBREVIATIONS

%	-	Per cent
@	-	At the rate of
C.D.	-	Critical difference
cm	-	Centimeter (s)
Conc.	-	Concentration (s)
Dia.	-	Diameter
<i>et al.</i>	-	and others
<i>etc.</i>	-	Etcetera
Fig.	-	Figure (s)
ha.	-	Hectares (s)
hrs.	-	Hours
<i>i.e.</i>	-	That is
m	-	Meter (s)
mm	-	Millimeter
NA	-	Nutrient Agar
No	-	Number (s)
°C	-	Degree celsius
PDC	-	Per cent Disease Control
PDI	-	Per cent Disease Intensity
PI	-	Per cent Incidence
ppm	-	parts per million
S.E.	-	Standard error
sp.	-	Species
T	-	Treatment
<i>viz.,</i>	-	Videlicet (namely)
<i>Xam</i>	-	<i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>
YGCA	-	Yeast Glucose Chalk Agar



Introduction



CHAPTER-I

INTRODUCTION

Cotton (*Gossypium* spp.) is the most extensively cultivated commercial crop and is a major fibre crop of global importance and has high commercial value. Cotton locally known as “White Gold” is also a king of cash crop.

Cotton is grown commercially in the temperate and tropical regions of more than 70 countries. Specific areas of production include countries such as China, USA, India, Pakistan, Uzbekistan, Turkey, Australia, Greece, Brazil, Egypt etc. where climatic conditions suit the natural growth requirements of cotton, which includes periods of hot and dry weather and adequate moisture obtained through irrigation.

Cotton is mainly grown for fibre needs of the human. Cotton almost accounts 65 per cent of fibre production in India. Edible oil is extract from cotton seed and de-oiled cakes are used as a cattle feed, which is a good source of high quality protein for animals. Cotton cake after extraction of oil is used as a good organic manure, which contains near about 6 per cent nitrogen, 3 per cent phosphorus and 2 per cent potash.

India is the only country in the world where all the four cultivated species of cotton, viz., *G. hirsutum*, *G. arboreum*, *G. herbaceum* and *G. barbadense* are cultivated on commercial scale, besides their hybrid combinations. The diversity of cotton cultivars and cotton agroclimatic zones in India are considerably larger as compared to other major cotton growing countries in the world.

India has the largest cotton area in the world with about 115.53 lakh hectares under cultivation, accounting for one-fourth of the global cotton area and has emerged as the world’s second largest cotton producer in 2017-18. China is the world’s leading cotton producer. It has been estimated that cotton contributes to approximately 30% of the Indian agricultural gross domestic product and considerable export earnings (Anonymous, 2017).

In India, the states of Maharashtra (26.63%), Gujarat (17.96%), Andhra Pradesh (13.75%) and Madhya Pradesh are the leading cotton producing states since these states have a predominantly tropical wet and dry climate. Area under cotton in Maharashtra is 38.72 lakh ha and production is around 78.25 lakh bales with an average productivity of 360.80 kg / ha (Anonymous, 2017).

Amongst the several factors responsible for reduction in yield and quality deterioration of cotton in India, diseases plays a vital role. Cotton crop in India is known to suffer from number of diseases. Important diseases of cotton are given in the following.

Fungal diseases:

Wilt	- <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>
Verticillium wilt	- <i>Verticillium dahliae</i>
Alternaria blight	- <i>Alternaria macrospora</i>
Grey mildew	- <i>Ramularia areola</i>
Anthracnose	- <i>Colletotrichum gossypii</i>
Ascochyta blight	- <i>Ascochyta gossypii</i>

Bacterial diseases:

Bacterial blight	- <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>
Crown gall	- <i>Agrobacterium tumefaciens</i>

Viral diseases:

Cotton leaf curl virus (CLCuV)

Of these, bacterial blight is one of the most important diseases of cotton and was first reported from Alabama (USA) by Atkinson in 1891. The disease was first reported in India from Rajapalayam, Tamil Nadu in 1918.

Xanthomonas axonopodis pv. *malvacearum* causing bacterial blight in cotton belongs to the kingdom– Bacteria, Phylum- Proteobacterium, Class– Gamma Proteobacteria, Order– Xanthomonadales, Family–

Xanthomonadaceae, Genus–*Xanthomonas*, Species–*axonopodis*, Binomial name – *Xanthomonas axonopodis*.

The genus *Xanthomonas* is a diverse and economically important group of bacterial pathogens, belonging to the gamma-subdivision of the Proteobacteria. Bacterial blight, caused by *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye, [which is the same as *Xanthomonas axonopodis* pv. *malvacearum* (Vauterin *et al.*, 2000)] can be a serious disease in most upland cotton (*Gossypium hirsutum* L.,) growing areas of the world (Bayles and Verhalen, 2005).

Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* is one of the serious diseases of cotton. It is recorded in almost every country in the world which grows cotton. It is an important disease of cotton in India, Pakistan, China, South East Asia, South America, Australia and Europe (Verma, 1986; Hillocks, 1992).

In India, it is an economically important disease in almost all the states wherever cotton is grown viz., Andhra Pradesh, Haryana, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu and Western Uttar Pradesh (Verma, 1986; Srinivasan, 1994).

Bacterial blight, although, is considered a parenchymatous disease, but it has now been shown that the organism penetrates vascular bundles. It is also considered as semi-systemic disease. Pathogen infects all the stages of plants and according to plant parts affected and symptoms produced, disease is recognized in four phases viz., angular leaf spot (on leaves), black arm lesions (on stem and petioles), boll rot and gummosis (on bolls) and seedling blight (on seedling) (Verma, 1986).

Bacterial blight causes severe losses because it reduces photosynthetic activity by destroying the chlorophyll content in leaves and stem. These disease has been found to cause losses in seed cotton yields to the extent of 50% (Simpson, 1956). Moreover it causes yield loss of 40.19 per cent (Bhattiporlu,

2013). Losses are less when only leaves are infected but when stem lesions are formed, the losses may be high as upto 90%.

The pathogen is both externally as well as internally seed borne (Verma and Singh, 1974), which plays important role in primary spread of disease. It has been also reported that host debris and infected soil help in survival of the pathogen from one season to another season (Srinivasan, 1994). Volunteer plants can be source of inoculum so also the weed hosts found nearby to cotton field (Verma, 1986; Srinivasan, 1994).

Therefore, considering importance of bacterial blight disease of cotton, the present studies were planned and undertaken at the Department of Plant Pathology, VNMKV, Parbhani during 2017-18, with following objectives

Objectives

1. Survey of bacterial blight of cotton in Nanded and Parbhani districts.
2. To isolate and prove pathogenicity of bacterial blight of cotton.
3. *In vitro* evaluation of different antibiotics along with fungicides against *X. axonopodis* pv. *malvaceum*.
4. Field evaluation of different antibiotics along with fungicide against *X. axonopodis* pv. *malvaceum*.
5. To study the host range of *Xanthomonas axonopodis* pv. *malvaceum*.

A decorative green vine with leaves and red flowers is positioned on the left side of the page, extending from the bottom towards the top. The vine has several leaves and two prominent red flowers with white centers. The title "Review of Literature" is written in a purple, cursive font to the right of the vine.

Review of Literature

A decorative green vine with leaves and red flowers is positioned on the right side of the page, extending from the bottom towards the right. The vine has several leaves and two prominent red flowers with white centers.

CHAPTER -II

REVIEW OF LITERATURE

Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* is one of the destructive diseases affecting cotton. Recent and important literature citations related to survey, isolation and pathogenicity, *in vitro* evaluation of the antibacterial chemicals, *in vivo* evaluation of the antibacterial chemical and host range are compiled and presented under appropriate headings and sub-headings in this chapter.

2.1. General about bacterial blight of cotton

Bacterial blight of cotton was first recorded in Alabama, USA in 1891 (Atkinson, 1891) but in India it was observed for the first time from Madras in 1918 (Ballard and Norris, 1923; Ramakrishanan and Ramkrishnan, 1950).

Patel and Kulkarni (1948) reported that bacterial blight of cotton was introduced in India during the middle of the 19th century through the exotics. In the 1940's disease was observed in the severe form in the rainfed cotton (*G. arborium* and *G. herbaceum*) (Uppal, 1948).

Bacterial blight of cotton has been found to cause economic losses in seed cotton yields to the extent of 50 per cent (Simpson, 1956). In India, estimates of losses was often upto 30 per cent (Meshram *et al.*, 1988; Mishra *et al.*, 2001; Patil *et al.*, 2003; Rampandu *et al.*, 1979). Bacterial blight affects both irrigated and rainfed cottons. According to Srinivasan (1994), irrigated cotton suffer heavy losses in years favourable to the disease. *Gossypium barbadense* is more severely affected than *G. hirsutum*. The irrigated winter Combodia cotton and the irrigated summer cottons (*G. hirsutum*) in Tamil Nadu often suffer severe losses. The irrigated cotton (*G. hirsutum*) of the Punjab, Haryana, Rajasthan and Uttar Pradesh are also subjected to heavy losses. Among the rainfed crops, the Karunganni cotton (*G. arboreum*) in Tamil Nadu, the herbaceums of Karnataka, Madhya Pradesh and the Westerns (*G. herbaceum*) of the Andhra Pradesh were badly affected in 1946-47 with

stem and boll attack (Uppal, 1948). Bacterial blight causes yield loss of 38.78 per cent (Bhattiporlu, 2013). Losses are less when only leaves are infected but when stem lesions are formed, the losses may be high as upto 90%.

2.1 Survey of Bacterial blight of cotton

Akello and Hilliock (2003) conducted surveys in 1995 and 1996 and found that bacterial blight *X. axonopodis* pv. *malvacearum* occurs throughout the main cotton growing areas of Uganda causing seedling blight angular leaf spot and bacterial boll rot. Black arm spread from leaves to stem, causing loss of fruiting branches.

Govindappa *et al.* (2008) conducted survey in areas of Dharwad, Haveri, Belgaum, Bagalkot, Bellery, Gahaeg, Raichur and Gulbarga districts of North Karnataka to assess the incidence of bacterial blight of cotton on farmer field and Agriculture Research Station. Bacterial blight was only in Dharwad districts of ARS Dharwad farm and MARS Dharwad recorded 25 % of 5% bacterial blight incidence. Haveri district recorded 10% bacterial blight incidence.

Jagtap *et al.* (2012) conducted extensive roving survey in different districts of Marathwada region of Maharashtra State to isolate the pathogen associated with the bacterial blight disease of cotton. In all 76 cotton fields were surveyed and average disease incidence (PI) to the tune of 51.12 per cent was observed. Highest disease incidence was noticed in Parbhani district (67%) followed by Hingoli (63%), Nanded (58%) and Latur (54%). The lowest disease incidence noticed in Jalna district (36%). Highest disease severity was noticed in Parbhani district followed by Hingoli, Latur, Nanded and Aurangabad.

Patole *et al.* (2016) reported that among the various diseases occurring on cotton, the foliar disease bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum* is gaining more importance in recent years because of their increasing incidence. This has been known to occur on all the various cultivated and wild species of cotton in Maharashtra.

Prashant *et al.* (2017) conducted a roving survey on cultivars' field during the crop season and a fix point survey on cotton periodically at Research farm. On Research farm, periodical observations were recorded on G. Cot. Hy. 12 which revealed that bacterial blight appeared during the 2nd week of July (2.0 %) and then gradually developed and reached at its peak (23.5 % PDI) during the 3rd week of September and then declined, but prevailed up to 1st week of December. Observations were also recorded on other cultivars. The susceptible cultivars viz., LRA 5166 showed bacterial blight intensity to the tune of 2.5 to 23.0 per cent. Moreover, non Bt cotton was more susceptible to the bacterial blight disease.

2.2. Isolation and Pathogenicity of bacterial pathogen

2.2.1. Isolation

Dyaneshwar *et al.* (1966) studied twenty eight species of *Xanthomonas* comparatively. All *Xanthomonas* species described by him were found to possess the true characteristics of the genus *Xanthomonas*. Some difference from the original description of *X. rubrilineans*, *X. maydis*, *X. jasminii*, *X. translucens* were recorded by them. Such minor difference between different isolates were not uncommon i. e. identical in morphological, cultural and physiological characters.

Akhtar and Khan (1988) isolated *Xanthomonas axonopodis* pv. *malvacearum* from typical lesions of blighted cotton.

Supriadi *et al.* (1996) isolated bacterial pathogen *Xanthomonas axonopodis* pv. *malvacearum* from leaves of cotton showing typical bacterial blight.

Jyoti *et al.* (2005) studied the bacterial blight disease of pomegranate and proved pathogenicity of *X. axonopodis* pv. *punicae*.

Hasan *et al.* (2008) collected the infected plant parts and plated on nutrient agar medium and isolated the yellow *Xanthomonas* colonies.

Dezordi *et al.* (2009) studied on semi-selective culture medium for *Xanthomonas axonopodis* pv. *malvacearum* and the isolated bacteria showed circular, yellow, mucoid and bright colonies.

Yenjerappa (2009) isolated the bacterium by employing the serial dilution plating technique using nutrient agar medium and yielded well separated, typical, yellow, mucoid, colonies of bacterium on nutrient agar medium after 72 hours of incubation at 30°C. For proving pathogenicity, the bacterial cell suspension (5×10^6 cfu /ml) of each of the isolates collected from different regions during survey were sprayed on to the susceptible pomegranate plants of Bhagwa variety. The characteristic symptoms were observed on pomegranate leaves after ten days of inoculation. Re-isolations were carried out from these lesions for each isolate and comparisons were made with the original culture to confirm the identity of the pathogen.

Madani *et al.* (2010) isolated thirty-seven isolates from diseased cotton plants formed yellow, convex, round and mucoid colonies on yeast dextrose agar medium.

Razaghi *et al.* (2012) collected infected leaves and stems with angular spots of Sahel and Acala cultivar and isolated yellow coloured colonies from cultured nutrient agar medium.

Hamid *et al.* (2012) collected typical symptoms of bacterial blight and the bacterium was isolated by dilution plate technique. The isolated bacterium produced yellow and round colonies on nutrient agar.

Jagtap *et al.* (2012) studied the bacterium (*Xanthomonas*) isolated from the diseased leaves on nutrient agar medium by streaking method. The isolated colonies were glistering with smooth, profused and mucoid growth.

Sain and Gour (2013) isolated the bacteria from infected soybean plant leaves and stem parts on NA medium. Yellow, circular and semi transparent colonies appeared.

Hasan *et al.* (2014) isolated the bacterium *X. axonopodis* pv. *malvacearum* that showed yellow, circular to round, viscous and mucoid colonies on nutrient agar medium.

Haq *et al.* (2015) isolated the bacteria from diseased bolls which showed smooth, translucent colonies with convex margins, yellowish pigmented colonies on nutrient glucose agar medium.

Khabbaz *et al.* (2017) collected 21 bacterial isolates during a survey from seven different cotton growing areas representing three districts of Tamil Nadu, India, only eight bacterial isolates of *Xanthomonas axonopodis* pv. *malvacearum*.

2.2.2. Identification

According to Bradbury (1984) cells of *Xanthomonas* were straight rods usually within the range 0.4 – 0.7 wide X 0.7 – 1.8 μ m long. They were Gram negative, aerobic and motile by a single polar flagellum. The optimum temperature for growth is usually 25-30 °C. Colonies were yellow, smooth and butyrous or viscid.

McGuire *et al.* (1986) identified six isolates as *Xanthomonas campestris* pv. *vesicatoria* on the basis of morphological and biochemical tests. All the isolates were Gram-negative, rod shaped, motile, aerobic, oxidase negative, catalase positive and amylotic positive.

Goto (1992) reported that bacterial cells are positive for hydrolysis of starch, aesculin, casein, liquefaction of gelatine and production of tyrosinase, catalase and reducing substances from glucose and hydrogen sulfide. The bacterium is negative for nitrate reduction, indole production and methyl red test.

According to Schiegl (1995) the genus *Xanthomonas* is classified under the family *Pseudomonodaceae* and the yellow-pigmented plant pathogen of this family have been unified in this genus.

Malavolta *et al.* (1999) carried out biochemical, cultural, physiological test and identified the pathogen as *Xanthomonas campestris*.

Carrillo *et al.* (2001) characterized and identified the bacterial isolates. Each isolate was evaluated for the Gram reaction, flagella staining and for response to biochemical tests like starch hydrolysis and catalase production .

El-Sadek *et al.* (2001) showed that all isolates were yellow-pigmented colonies characterized by rod-shaped, non-sporulating and motile Gram negative bacteria. Based on morphological, physiological and biochemical characteristics, the bacterial isolates were identified as *Xanthomonas vesicatoria*.

Bobosha (2003) isolated forty-eight different bacterial isolates and tested for their gram staining reaction and for their incapability of utilizing asparagines as a sole source of carbon and nitrogen. Isolates with negative gram staining reaction and those that did not grow on asparagine medium were subjected to biochemical, hypersensitivity and pathogenicity test.

Srabani (2003) characterized that the bacterium was gram negative, rod shaped and appeared singly. Single bacterial colonies were round, flat and bright yellow in nutrient agar and turbid yellow in nutrient broth. The bacterium showed positive reaction to starch hydrolysis and gelatine liquefaction. The pathogen showed positive reaction for catalase but negative for oxidase reaction. The bacterium did not reduced nitrate, produced hydrogen sulphide and fermented lactose and sucrose.

Mitrev and KovacEvic (2006) conducted biochemical tests and presented that all investigated bacterial strains were gram negative, aerobic, catalase- and amino peptidase- positive, oxidase and urease negative. They hydrolyzed aesculin, gelatin, Tween 80 but did not hydrolyzed starch.

Dhutraj and Gokhale (2007) conducted an experiment to ascertain the effect of staining reaction, morphological, cultural and physiological characters on seed borne *Xanthomonas campestris* pv. *vignae radiata*. According to the

Bergey's manual of determinative bacteriology, organism was found *Xanthomonas campestris* pv. *vignaeradiata* of mungbean.

Kavitha and Umesha (2007) isolated the pathogen *Xanthomonas vesicatoria* and further the pathogen was confirmed by biochemical and physiological tests.

Hasan *et al.* (2008) stated that the studied bacterium *X. axonopodis* pv. *malvacearum* was an aerobic (catalase positive), mobile, gram-negative, copiously mucoid and wet, non spore forming rod. Colonies varied from pale yellow to yellow, depending on the race and medium, convex and smooth, with entire margins.

Dhakal *et al.* (2008) carried out detailed study to isolate the pathogen and confirm it by available biochemical tests and pathogenicity test. The isolated pure culture was subjected to gram staining, catalase test, oxidase test, O-F test, starch hydrolysis, nitrate reduction test, methyl red test, Voges-Proskauer test, indole production test, urease test and carbohydrate utilization test.

Dezordi *et al.* (2009) characterized the *X. axonopodis* pv. *malvacearum* by conducting morphological, physiological and biochemical characters. The reaction showed positive for KOH, catalase test, hydrolysis of starch, hydrolysis of gelatin, hydrolysis of esculin, lipolysis of Tween and the reaction showed negative for reduction of nitrate and hydrolysis of casein.

Opara *et al.* (2009) conducted the cultural, physiological and biochemical analysis and showed that the bacteria were gram negative, yellow, aerobic, rod shaped with a polar flagella. The bacteria colonies exhibited strong starch hydrolysis, metabolized glucose and produced acid from arabinose, sucrose and cellobiose but not from ducitol or sorbitol. Based on bacteriological characteristics, the bacteria strains were identified as *Xanthomonas campestris* pv. *vesicatoria*.

Lamichhane *et al.* (2010) reported that yellow pigmented bacterial colonies were consistently isolated from diseased tissues and were identified as *Xanthomonas vesicatoria* based on morphological and biochemical tests. The bacterium were gram-negative, rod shaped, motile, aerobic, oxidase negative, catalase positive and amylolytic positive.

Madani *et al.* (2010) identified thirty-seven isolates as *Xanthomonas axonopodis* pv. *malvacearum* from biochemical and physiological tests. Cells were gram negative, obligate aerobes and utilised glucose oxidatively. All isolates produces H₂S from cysteine and formed levan.

Ogunjobi *et al.* (2010) worked on physiological studies of *Xanthomonas axonopodis* pv. *manihotis* strains isolated in Nigeria and reported that bacteria showed positive reaction to citrate utilization test and negative to indole production test.

Sujatha and SaiGopal (2010) reported that citrus canker was caused by *Xanthomonas axonopodis*. The pathogen was isolated from the infected fruits of citrus orchards in the present study. It was characterized by preliminary studies and identified as gram negative rod shaped organism. Bacteria was positive to gelatine utilization test, catalase test, methyl red test and negative to casein hydrolysis and indole test.

Ajayi *et al.* (2011) studied the different physiological properties of *Xanthomonas* spp. and found that bacteria showed positive reaction for indole production test, citrate utilization test and amylase production test.

Hamid *et al.* (2012) showed that the bacterium *Xanthomonas campestris* pv. *malvacearum* was rod shaped and motile with single polar flagellum. The bacterium indicated gram-negative reaction.

Rukhsana *et al.* (2012) showed that isolated *Xanthomonas oryzae* pv. *oryzae* were yellow, circular, smooth, convex and viscous bacterial colonies on Yeast Extract Glucose Chalk Agar (YGCA). Gram staining demonstrated that pathogen was a gram negative rod shaped. KOH test they performed in order to

confirm gram staining results and confirmed that they are gram negative. The bacterium was oxidase negative and starch hydrolysis positive.

Raghuwanshi *et al.* (2013) showed that the isolated bacteria tested positive for KOH test and negative for gram staining indicating the gram negative nature of bacteria. The bacteria were positive for oxidase test, catalase test, starch hydrolysis, gelatin liquefaction and lipid hydrolysis.

Balan *et al.* (2014) conducted morphological and biochemical characters of *Xanthomonas* and the results obtained indicated that the bacteria produced acid oxidatively from sucrose, glucose, maltose, lactose, dextrose, fructose, mannose and arabinose whereas no acid was produced from dulcitol, inositol, adinitol and sorbiton. It produced H₂S, liquified gelatine and hydrolyzed starch. It utilized citrate, produced lipase and ammonia, whereas it showed negative reaction for methyl red and urease test.

Haq *et al.* (2015) worked on biochemical characteristics of bacterium. The result showed that the reaction of gram staining and 3% KOH confirmed bacterium as gram negative. The reaction was positive to Voges-proskauer reaction, Ornithine decarboxylase, acid production to D-cellobiose, lactose, maltose, raffinose, sucrose, salicin. The reaction was negative for acid production to melibiose, adonitol, dulcitol, sorbitol and to α -Methyl-D-glucoside. Indole production, arginine hydrolase and lysine decarboxylase were negative to isolated bacterium.

2.2.3. Pathogenicity

Akhtar and Khan (1988) characterized the bacterium and confirmed the pathogenicity on three cotton cultivars.

Supriadi *et al.* (1996) isolated bacterial pathogen from leaves of cotton showing typical bacterial blight, following isolation and purification, two isolates, T 868 and T 873, were tested for their pathogenicity.

Vincente *et al.* (2001) inoculated the bacterial suspension on the three youngest leaves on each plant and proved the pathogenicity by describing the typical symptom.

Das and Verma (2003) collected thirty samples of bacterial blight and studied for pathogenicity. Pathogenicity study of selected bacterial isolates showed that out of 30, 10 were *X. axonopodis* pv. *malvacearum* and 20 were saprophytic phylloplane bacteria.

Manjula *et al.* (2003) proved the pathogenicity of *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight of pomegranate. Isolations from the affected leaves and fruits formed yellow coloured, convex, round, shiny colonies on nutrient agar medium. These isolates when inoculated on pomegranate plants, produced typical symptoms of the disease as minute water soaked lesions which later turned brown and surrounded by diffused water soaked zone or yellow halo on both fruits and leaves

Petersen *et al.* (2007) observed symptoms which included leaf and fruit spots, and cankers on stems, branches and trunks of pomegranate. Based on biochemical and molecular analysis and pathogenicity tests, the bacterium *Xanthomonas axonopodis* pv. *punicae* was identified as the causal agent. This was the first report of bacterial blight on pomegranate in South Africa.

Hasan *et al.* (2008) infiltrated the cultivars with a suspension of selected bacterial isolates prepared by suspending colonies in ddH₂O to a concentration of 10⁷ CFU/ml and proved the pathogenicity.

Madani *et al.* (2010) inoculated the seedlings of cotton cultivar by injecting the bacterial suspension with a syringe, needle and proved the pathogenicity.

Mogle *et al.* (2009) tested the pathogenicity of *X. axonopodis* pv. *punicae* on young and healthy plants of highly susceptible pomegranate cultivar Ganesh grown in earthen pots in polyhouse. Forty days old healthy leaves of pomegranate were inoculated with sterilized thorny seeds of

Xanthium strumarium and nodes by leaf cut method. They stated that typical symptoms of disease were appeared on the leaves within 9 to 13 days after inoculation with irregular water soaked spots encircled with yellow halo.

Razaghi *et al.* (2012) proved the pathogenicity in which all selected strains was induced typical angular leaf spot on treated leaves of Varamin and Sahel cotton cultivar.

Jagtap *et al.* (2012) inoculated the leaves with the homogenized culture of *Xanthomonas*. They described the symptoms appeared and proved the pathogenicity.

Sain and Gour (2013) inoculated the purified bacterium on soybean plants. They noted the typical symptoms and proved the pathogenicity.

Hasan *et al.* (2014) assessed the bacterial blight of cotton through some dispersal mechanism of *X. axonopodis* pv. *malvacearum* and proved the pathogenicity by toothpick scratch method.

Bora and Kataki (2014) established the pathogenicity test by spraying 48 hour old bacterial culture suspension (2×10^8 cfu/ml) on pinpricked 40 days old healthy pomegranate leaves. Infection occurred within 23 days of inoculation and produced identical symptoms observed on original pomegranate plant. The organism was re-isolated from artificially inoculated plant, which yielded an organism similar to one used in the inoculation experiments. This satisfied all the conditions required to establish that the disease was bacterial borne disease which was caused by the pathogen *Xanthomonas axonopodis* pv. *punicae*.

Haq *et al.* (2015) inoculated the bacterial suspension on bolls by injection method using syringe and proved the pathogenicity.

Khabbaz *et al.* (2017) during a survey collected 21 bacterial isolates from seven different cotton growing areas representing three districts of Tamil Nadu, India, only eight bacterial isolates of *Xanthomonas axonopodis* pv. *malvacearum*.

2.3. Symptoms of bacterial blight of cotton

2.3.1. Symptoms on leaves

Verma (1986) described the symptoms of bacterial blight of cotton as initial minute lesions which were water soaked and scattered on the under surface of the leaves. The lesions gradually increased in the size (upto 5 mm dia.), first turned brown then black, and formed angular dead areas with reddish or purplish border bounded by veinlets. The lesions also became visible on the upper surface of the leaves. When the lesions extended along the edges of the veins the symptom was described as vein blight or black vein. He also mentioned that bacterial ooze was often noticeable in the form of crust on the lower surface of the leaves in severe infection. Later on several of the lesions coalesced to form very large spots giving the appearance of the blight. Such leaves generally died and were shedded.

Mehrotra (1988) mentioned that in the bacterial blight of cotton, on the leaves the spots were usually angular and first appeared on the under surface and then on the upper surface. The disease advanced along the main veinlets and veins to cause finger like lesions on leaf blade which was termed as vein blight. Vein blight leaves became crinkled and twisted inwards and dried. He mentioned that the infection may spread through the leaf blade to the petiole, causing severe defoliation.

Alipi (1989) studied survival of a *Xanthomonas axonopodis* pv. *malvacearum*, the cause of bacterial blight of cotton, in sterile and non sterile black earth soil. He found that the Pathogen had limited capacity for survival in soil free from cotton plant material.

Sheo-Raj and Verma (1989) described symptoms, pathogens, epidemiology and control of bacterial blight, *Alternaria* leaf spot, root rot and *Fusarium* wilt. Angular leaf spot, black arm, boll rot and seedling blight symptoms of bacterial blight described.

Swanepoel (1990) gave brief account of epidemiology, symptoms and control of bacterial blight caused by *X. axonopodis* pv. *malvacearum*. He described three forms of the disease, angular leaf spot, black arm and boll rot.

Hillocks (1992) described the symptoms which were more or less similar to those described by Verma (1986). In addition to this he described distortion and necrosis of leaf in blight phase of the disease.

Srinivasan (1994) described symptoms of bacterial blight of cotton which were similar to Hillocks (1992). In addition he also mentioned that the spots turned sooty coloured on *barbadense* cotton but were more often dark brown on *hirsutum* cotton.

Verma (1995) reviewed on bacterial blight of cotton, symptomatology, identification and distribution of races, pathogenicity, disease control. He identified four phases of disease recognized depending on plant parts affected i.e. angular leaf spot (leaf), black arm (stem), boll rot (boll) and seedling blight (seedling).

Chopra (1998) described symptoms of damage and control of the main cotton diseases in India, including bacterial blight, root rot and *Fusarium* wilt.

Koenning (2004) described that bacterial blight starts out as angular leaf spot with a red to brown border. The angular appearance is due to restriction of the lesion by fine veins of the cotton leaf.

Chidambaram (2007) described the symptoms of bacterial blight as dark green, water soaked, angular lesions of 1 to 5 mm across the leaves and bracts, especially on the under surface of leaves and hence called angular leaf spot. Sometimes extensive dark green, water soaked lesions along the veins appear known as vein blight. Symptoms are usually more prevalent on lower leaves than on upper leaves. Lesions dry and darken with age and leaves may be shed prematurely resulting in extensive defoliation.

Kirkpatrick (2011) mentioned that the bacterial blight pathogen can infect all parts of the plant. Symptoms of bacterial blight vary according to the

plant part that is infected and the stage of the crop when infection occurs. Early symptoms include small water-soaked spots on leaves that rapidly enlarge to take on an angular shape. Lesions may coalesce rapidly and follow the main leaf veins. At this point in the season, lesions on leaves and lesions on squares and bracts are likely present in fields.

Allen (2012) described that the symptoms of bacterial blight on leaves first appear as water-soaked spots that develop into angular-shaped lesions delimited by veins. In severe cases, bacterial blight infection in susceptible varieties results in defoliation and a reduction in plant height. During elongated periods of environmentally conducive conditions, large yield reductions in addition to poor fiber quality can be observed. Depending upon the level and timing of infection and prevailing environmental conditions, secondary spread of the pathogen can occur either naturally or mechanically.

Allen (2014) described the symptoms of bacterial blight as expression on individual plant parts. Lesions on leaves appear as small, angular spots that in some cases can congregate around the veins. The angular nature of the spots are produced by the capillary veins limiting the spread of the bacterium within leaf tissues. By flipping the leaf over and observing the underside of the leaf the water-soaking around the spot will develop a greasy appearance, especially in the presence of morning dew. He added that if having trouble determining bacterial blight from other diseases (or even herbicide injury) look for two specific characteristics: 1) a systemic vein infection, generally in the mid to upper canopy and 2) the appearance of extreme water-soaking around lesions that may congregate on leaf tissues. Lesions on bracts look more elongated than on leaves and are generally brown to maroon in color.

2.3.2. Symptoms on stem and petioles

Symptoms on petioles and stem as black arm stage of bacterial blight of cotton have been described by many workers (Verma, 1986; Hillocks, 1992; Srinivasan, 1994). The commonly described symptoms by these workers

included appearance of elongated, greyish to sooty black lesions on petioles and stems and oozing out of bacterial slime under favourable environmental condition. Deep cracks were reported on stem, which were liable to break off during strong wind causing considerable losses. The black arm phase was considered as most serious phase of the disease, because it resulted in the complete loss of plants and yield.

Koenning (2004) described that spots of bacterial blight disease on infected leaves may spread along the major leaf veins. As disease progresses, leaf petioles and stems may become infected resulting in premature defoliation. Black cankers may girdle the stem or branches causing the portions to die above the canker. A white waxy crust containing the bacterium may form on old leaf spots or cankers.

Chidambaram (2007) described the symptoms of bacterial blight of cotton as black lesions on the stem which girdle and spread along the stem or branch known as black arm.

Kirkpatrick (2011) mentioned that the bacterial blight pathogen can infect all parts of the plant. In very susceptible varieties, or where the disease is severe, lesions may form on petioles and stems, and leaf defoliation may occur.

Allen (2014) described the symptoms of bacterial blight as expression on petioles – the “black-arm phase” of the disease can occur on petioles. Dark-colored, ashen lesions generally surround the entire petiole.

2.3.3. Symptoms on seedlings

Infection of bacterial blight arising from the seed results in seedlings with dull green flaccid areas extending from the periphery of the cotyledons (Wickens, 1956). Symptoms described by earlier workers (Verma, 1986; Hillocks, 1992; Srinivasan, 1994) include development of lesions on the stem and cotyledons. The first symptom was reported to appear on the margins of the ventral side of the cotyledons. These appear as small water soaked, circular

or irregular spots (not angular). The lesions turn black or brown and the cotyledons shrivel and distorted. From the cotyledons the expanding lesion passes on to the young stem and from this point, the seedling may be affected by the secondary infections upto the apex and may often die. Such extensive damage constitute seedling blight. If the seedling survives, the infection may spread to the other growing parts of the plant.

2.3.4. Symptoms on bolls

Symptoms of bacterial blight on bolls have been reported by several workers (Verma, 1986; Hillocks, 1992; Srinivasan, 1994). These workers termed symptoms on bolls as boll lesions, boll rot, blight or gummosis. The basal infection of the flower buds and young bolls has been reported to cause premature boll shedding. On the bolls, first symptom observed was small, round, water soaked raised spots. As the disease advanced the lesions became irregular in shape and turned brown black as well as depressed in the centre. The lesions sometimes covered the entire boll and even penetrated in the internal parts of boll. Sunken black lesions when dry are termed as boll blight. Due to invasion of secondary microorganisms infected bolls may rot and it is also known as gummosis. Infected bolls dehiscing prematurely where the lint is discoloured and weak. The pathogen may also infect seed externally and internally.

Koenning (2004) described that bolls may become infected due to bacterial blight causing boll rot which results in rotted seed and discolored lint. Infected bolls have round, rather than angular, lesions that initially may appear water-soaked. As infection proceeds, bolls lesions will be sunken and dark brown or black.

Chidambaram (2007) described the symptoms of bacterial blight as dark green, water soaked, greasy, circular lesions of 2 to 10 mm across the bolls, especially at the base of the boll under the calyx crown. As the boll matures the lesions dry out and prevent normal boll opening. This phase of symptom is called as Boll rot.

Kirkpatrick (2011) described that as bolls develop, water-soaked lesions may form due to bacterial blight disease. These lesions are probably of the most concern from a yield loss standpoint because they will result in stained lint and provide entry for secondary boll rot pathogens.

Allen (2014) described the symptoms of bacterial blight expression on bolls –the presence of the small, water-soaked lesions on bolls. Lesions on bolls will go from having a round water-soaked appearance to a dark, black appearance when the lesions are most mature.

2.5. *In vitro* evaluation of chemicals against *X. axonopodis* pv. *malvacearum*.

2.5.1. Chemicals

Thirumalachar *et al.* (1956) and Thirumalachar (1968) observed effectiveness of aereomycin, terramycin and chloramfenicol at 60 ppm and NA penicillin at 250 to 500 ppm against various strains of *X. axonopodis* pv. *malvacearum*.

Verma *et al.* (1975) observed that better inhibitory concentrations of streptomycine sulphate, agrimycin, blitox and copper oxychloride under *in vitro* conditions against *X. axonopodis* pv. *malvacearum*.

Wu *et al.* (1980) studied the *in vitro* efficacy of chemicals against *Xanthomonas axonopodis* pv. *mangiferae indicae* found that streptomycin, dikar, dithane M-22 and hinodan were inhibitory to growth of pathogen.

Nath *et al.* (1981) determined *in vitro* effects of antibiotics and fungicides on *X. axonopodis* pv. *malvacearum*. Organomercurials followed by trichlorophenol were the most effective.

Shah *et al.* (1991) tested chemicals for their efficacy against bacterium and found streptocycline gave best control followed by streptocycline + copper oxychloride.

Khan (1995) observed that multiplication of *X. axonopodis* pv. *malvacearum* on nutrient agar amended with agrimycin-100 was significantly inhibited at concentration of 5, 15, 30, 45 and 60 ppm.

Manjula *et al.* (2003) reported that Paushamycine was highly effective against Bangalore leaf isolates of *X. axonopodis* pv. *punicae* at 500 ppm producing inhibition zone of 19.00 mm and also Streptocycline at same concentration was highly effective by producing 25.6 mm inhibition zone.

Singh *et al.* (2007) tested the efficacy of 12 fungicides and two antiseptics by disc plate method against *Xanthomonas*. Thiram, mercuric chloride, carbendazim + mancozeb, bavistin, captan and formaldehyde gave good results in ascending order.

Genaet *al.* (2008) studied the efficacy of agrochemicals against *Xanthomonas axonopodis* pv. *vignicola* and revealed that maximum inhibition was obtained by labisrtyl @ 300 ppm (16.50 mm) followed by streptocycline @ 300 ppm (16.20 mm), plantomycin @ 300 ppm (15.80 mm), erocin-250 @300 ppm (12.77 mm) and blitox50% WP (9.40 mm).

Kumar *et al.* (2009) studied the toxicity of chemicals at respective concentration against *Xanthomonas oryzae* pv. *oryzae* by paper disc method and found that the chemicals copper oxychloride (0.25%) + Streptomycin sulphate (200 ppm) were most effective in controlling the disease.

Raut *et al.* (2010) evaluated bioagents, botanicals and chemicals against bacterial blight of cotton and among these copper hydroxide (19.67 mm) was found effective in inhibiting the *X. axonopodis* pv. *malvacearum* followed by copper oxychloride + streptomycin sulphate. Bioagents *Pseudomonas fluorescens* Pf-1 and neem seed extract was also found effective.

Jagtap *et al.* (2012) studied efficacy of different chemicals *in vitro* against the *X. axonopodis* pv. *malvacearum* found that the maximum inhibition was by copper oxychloride 0.25% + streptocycline 100 ppm (18.33 mm) followed by copper oxychloride 0.25% + agrimycin 100 ppm (14.33 mm).

Ehetisham-ul-haq *et al.* (2013) studied *in vitro* evaluation of three chemicals against colony growth of *X. axonopodis* pv. *malvacearum*. Plant protector chemical was found effective at 600 ppm.

Raghuwanshi *et al.* (2013) studied the effect of different chemicals under *in vitro* condition by using poison food technique. Complete control in all four isolates was observed with Bordeaux mixture 1%; captan (0.25%) + copper oxychloride (0.3%), captan (0.25%) + copper hydroxide (0.3%), bronopal (500 ppm) + copper oxychloride (0.3%), streptocycline (250 ppm) + copper hydroxide (0.3%), streptocycline (500ppm) +copper hydroxide (0.3%) during *in vitro* study.

Ambadkar *et al.* (2015) studied the efficacy of different antibiotics for management of bacterial blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. *In vitro* study revealed that antibiotic streptocycline had maximum inhibition zone of 22.21 and 31.60 per cent at 250 and 500 ppm concentrations against *X. axonopodis* pv. *punicae*, followed by tetracycline (18.26 and 27.53 %) and bacterinol (17.40 and 27.15 %) respectively.

Haq *et al.* (2015) studied the *in vitro* efficacy of antibiotics against *Xanthomonas axonopodis* pv. *malvacearum* and found that streptomycin sulphate(1.10 cm) at 1000 ppm was the significant dose against bacterial growth followed by oxytetracycline.

Ashish *et al.* (2016) studied the effect of agro-chemicals on severity of bacterial blight and fruit quality in pomegranate. Three contact fungicides viz., Blitox (50 WP), Kocide (77 WP) and Bordeaux Mixture (2:2:250) and two antibiotics viz., Streptocycline and Bactrinashak (0.05%) at five different concentrations were evaluated for their efficacy against the growth of *X. axonopodis* pv. *punicae* by inhibition zone assay method. It was clearly indicated that among contact fungicides and antibiotics, maximum mean inhibition zone (cm) was achieved with fungicides rather than antibiotics. Blitox at concentration of 3000 ppm was found effective followed by Kocide at

concentration of 2500 ppm. Streptocycline was found effective at concentration >200 but less than 500 ppm. Bactrinashak proved least effective in inhibiting the growth of bacteria. Growth was also inhibited by Bordeaux mixture at any of the tested concentrations.

Antre *et al.* (2016) studied efficacy of total 18 the chemicals, botanicals and bioagents against *Xanthomonas axonopodis* pv. *punicae* by using disc diffusion method. Maximum zone of inhibition was recorded in chemical treatments. Copper oxychloride (0.3%) + Sreptomycin sulphate (500 ppm) was found significantly superior in inhibiting the growth of bacteria with 14.33mm zone of inhibition.

Thwipa *et al.* (2016) studied fourteen strains of antagonistic bacteria *Paenibacillus*, water extracts and oil of some plants (*viz.*, *Syzygium aromaticum*, *Nigella sativa*, *Trigonella*, *foenum*, *graecum* and *blubs* of *Allium sativum*) and camel urine were screened for their efficacy in inhibiting the growth of *Xanthomonas axonopodis* pv. *malvacearum* *in vitro*.

Sajid *et al.* (2017) evaluated plant extracts and chemicals against *X. axonopodis* pv. *mangiferae* it showed maximum inhibition zone of bacterial growth was expressed by Flare (1.693cm) at all concentrations followed by Plant Protector (1.473 cm), Mancozeb (1.290 cm), Agrimycine (1.150 cm) and copper oxy-chloride (0.953) cm respectively while in case of plant extracts maximum inhibition was expressed by *N. tabacum* (0.650 cm) followed by *A. indica* (0.486), *M. oleifera* (0.350), *D. alba* (0.256 cm) and *C. longa* (0.168 cm).

2.6. In vivo evaluation of chemicals against *Xanthomonas axonopodis* pv. *malvacearum*

2.6.1. Chemicals

Sharma *et al.* (1979) tested eight chemicals *in vitro* condition against *Xanthomonas versicatoria* by paper disc method and turbidimetric method. The studies revealed that combination of streptocycline and copper sulphate

was most effective but stable bleaching powder showed maximum inhibition when tested by Thompson's method.

Chauvan *et al.* (1983) stated that bacterial blight of cotton (*Xanthomonas campestris* pv. *malvacearum*) could be effectively controlled by spraying a mixture of Agrimycin (0.01%) + Blitox (0.2%) at three different stages of plant growth *i.e.* 40 to 45, 70 to 80 and 80 to 95 days after sowing.

Krishna and Nema (1983) tested five chemicals namely Bordeaux mixture (5: 5: 50), Pasuhamycine, Agrimycin-100, streptocycline and plantomycine against citrus canker. Among five chemicals streptocycline at 500 ppm with four sprays schedule was effective in reducing the disease during both years.

Akhtar and Khan (1988) worked on bacterial blight of cotton and its control using seed dressing and seedling sprays. The result indicated that the most effective seed treatment was sulphuric acid followed by mercuric chloride, agrosan 5W and Granosan M. The most effective spray was terramycin followed by penicillin, streptomycin, perenox and zerlate.

Singh and Singh (1988) conducted field trials at Ludhiana, during 1980-81 and 1981-82, under natural incidence of *Xanthomonas axonopodis* pv. *malvacearum* and found that the disease was effectively reduced by copper oxychloride alone or in combination with either streptocycline or plantomycin.

Patil *et al.* (1990) used three fungicides *viz.*, Dithane M-45 (0.25%) , Copper oxychloride (0.25%) and Bavistin (0.1%) either alone or in combination with streptocycline (50 ppm) against bacterial blight of paddy (*Xanthomonas campestris* pv. *oryzae.*). Three years field trial (1986-89) revealed that wet seed treatment with Streptocycline (100 ppm), one nursery spray, seedling dip at transplanting stage followed by two sprays after transplanting at interval has effective in reducing disease incidence significantly as compared to fungicides and bactericides alone.

Mishra and Om Prakash (1992) studied the control of bacterial canker of mango caused by *Xanthomonas campestris* pv. *mangiferae indicae* by testing five test chemical viz., Bavistin, Blitox, Bacterinol-100, Streptocycline and Paushamycine. Out of the five chemicals evaluated during 1987-88, Streptocycline 200 ppm (a.i. Streptomycin sulphate) gave the best control in both years followed by Bacterinol-100.

Chand *et al.* (1992) studied the efficacy of bactericides against bacterial canker disease of grapevine. Copper oxychloride 1500 ppm and 3000 ppm, Streptomycin sulphate 300 ppm and 500 ppm, bacterinol-100 at 500 ppm and 1000 ppm, Oxytetracycline 300 ppm and 500 ppm, Bordeaux mixture (3:3:50) and Bactosan were tested against bacterial canker of grapevine. Among the chemicals tested Copper oxychloride followed by Bordeaux mixture gave significant reduction in disease intensity.

Venkateshwarlu *et al.* (1992) tested the combination of mancozeb + copper oxychloride, streptomycin + tetracycline + copper oxychloride and Bordeaux mixture for their compatibility with two insecticides viz., monocrotophos and fenvalerate for the control of canker disease and leaf miner. Plots sprayed with streptomycin + tetracycline + copper oxychloride recorded least intensity of canker, followed by those treated with streptomycin + tetracycline + copper oxychloride + fenvalerate sprays which were found most effective for control of both leaf miner and canker disease of citrus.

Hussain and Tahir (1993) worked on chemical control of bacterial blight of cotton and found that the best control of *Xanthomonas axonopodis* pv. *malvacearum* on inoculated plants was given by agrimycin-100 (streptomycin + oxytetracycline) in combination with trimiltox (copper oxychloride). Treated plants showed the lowest disease index and highest yield of seed cotton compared with those receiving other test sprays.

Khan (1995) observed that the multiplication of *Xanthomonas axonopodis* pv. *malvacearum* on nutrient agar amended with agrimycin-100 was significantly inhibited at concentration of 5, 15, 30, 45 and 60 ppm.

Symptoms of bacterial blight were significantly controlled on inoculated plant at concentration of 30, 45 and 60 ppm.

Patil *et al.* (1997) studied chemical control of bacterial blight of cotton during 1988-89, 1989-90 and 1990-91 at Cotton Research Unit, PDKV, Akola. Amongst the treatments streptocycline + copper oxychloride was significantly superior over rest of the chemical treatments.

Upasana and Verma (2001) evaluated the effects of single or combined applications of Copper oxychloride (0.3 %), Bordeaux mixture (2.5:2.5:3), Captan (0.3%), Kavach (0.2%), Carbendazim (0.1%), Dithane M45 (0.3%), Areofungin (100 ppm) and Streptocycline (100 and 200 ppm) against black spot of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* in a field experiment in Ludhiana, Punjab during May 1997. They found that the intensity of the disease on fruits and leaves was lowest with 100 and 200 ppm Streptocycline application (18.50 and 13.47%)

Dreo *et al.* (2003) reported that the control of *Xanthomonas axonopodis* pv. *phaseoli* disease includes planting healthy seed, rotation with non-host plant and use of resistant cultivars. Decontamination of seeds is possible using chemical and physical treatment.

Islam *et al.* (2003) conducted a field experiment and studied effect of chemicals in controlling bacterial blight of cotton. The result revealed that seed treatment with streptomycin sulphate at 0.15% + foliar spray with cupravit at 150 ppm gave maximum seed germination percentage (86.31%) and the lowest disease index of 21.24% after 3 foliar sprays (at 104 days after sowing).

Gent and Schwartz (2005) worked on management of *Xanthomonas* leaf blight of onion under field condition and found that four weekly application of acibenzolar-S-methyl reduced the severity of *Xanthomonas* leaf blight as or more effectively than 9 to 12 weekly application of copper oxychloride.

Kumar *et al.* (2006) worked on management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* and the result

revealed that maximum yield and effective control of disease was achieved by application of bromopal (500 ppm) + copper oxychloride (2000 ppm) followed by streptocycline (500 ppm) + copper oxychloride (2000 ppm) with disease incidence of 19.08% and 19.65% respectively as compared to control 78.65% incidence.

Pathak and Godika (2006) studied on management of bacterial blight of cotton *Xanthomonas axonopodis* pv. *malvacearum* through chemicals and found that seed treatment with 100 ppm streptocycline for 2h followed by 2 sprays of 100 ppm streptocycline+0.3% copper oxychloride was the best treatment which was significantly superior over the control and other treatments.

Govindappa *et al.* (2008) evaluated the effect of propineb (0.1%), propineb (0.2%), propiconazole (0.1%), copper oxychloride (0.3%) + streptomycin sulphate (0.05%) against the foliar diseases. Of these copper oxychloride (0.3%) + streptomycin sulphate (0.05%) gave better control of the foliar disease viz., Bacterial blight, *Alternaria* blight and Grey mildew.

Raghavendra *et al.* (2009) evaluated the role of cultivars, physical, chemical and organic treatments in the management of bacterial blight of cotton and found that organic treatment exhibited better performance in increasing germination and in reduction of bacterial blight disease whereas in chemical treatment the incidence of blight was reduced to great extent and germination was effected.

Sharma *et al.* (2009) conducted field trial for management of bacterial blight and found that judicious sprays of antibiotic streptocycline (500 ppm) in combination with fungicides like carbendazim (0.15%) / mancozeb (0.2%) / copper oxychloride (0.25%) resulted in 82.2% disease control.

Suryawanshi *et al.* (2009) evaluated the efficacy of two antibiotics (Streptocycline and Bacterinol) and two fungicide (Carbendazim and copper oxychloride) against *Xanthomonas axonopodis* pv. *punicae*. They reported that both antibiotics and fungicides tested @ 250, 400, 500 and 1000 ppm

significantly inhibited growth of the test bacterium and significantly highest growth of 31.11 and 31.96 percent was recorded with Streptocycline respectively at 500 ppm and 1000 ppm. This was followed by the antibiotic Bacterinol (28.32% and 33.60% inhibition), fungicides Copper oxychloride (25.55%) and Carbendazim (28.32%). Both antibiotics and fungicides evaluated under field conditions were also found effective in reducing the oily spot disease intensity.

Rathore (2010) studied on efficacy of streptocycline and plant extracts against *Xanthomonas axonopodis* pv. *vignaradiatae* of green gram and the results indicated that minimum disease intensity (6.1%) was recorded by spraying streptocycline (0.01%) followed by garlic clove, mesquite leaf, onion bulb with the disease intensities of 11.2%, 13.3%, 13.9% respectively.

Ingole *et al.* (2011) worked on chemical and biological management of bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum*. The results indicated that foliar sprays of copper oxychloride @ 0.2 per cent + streptomycin sulphate @ 100 ppm were effective to reduce the bacterial blight incidence (37.41%), intensity (13.08%) with a maximum yield (1287 kg/ha) whereas, maximum disease incidence (57.59%), disease intensity (32.84%) and minimum seed cotton yield was recorded in untreated control.

Mondal and Mani (2012) reported the efficacy of nanocopper in suppression of growth as well as in the water soaked lesions induced by *X. axonopodis* pv. *punicae*. The nanocopper suppressed growth at 0.2 ppm, *i.e.* >10,000 times lower than that usually recommended for copper oxychloride. Scanning electron microscopy (SEM) revealed cell wall degradation in nano copper treated bacterial cells that failed to colonize plant tissue as well as to produce the characteristic intense water soaking. This study suggested that nano copper could be employed as an environmentally friendly strategy for the management of pomegranate bacterial blight.

Jagtap *et al.* (2012) worked on management of bacterial blight disease of cotton with chemicals. The results indicated that per cent disease incidence was

reduced by copper oxychloride 0.25% + streptocycline 100 ppm (19.36%) followed by carbendazim 0.1% + streptocycline 100 ppm (20.87%) and copper oxychloride 0.25% + agrimycin 100 ppm (22.47%).

Lokesh *et al.* (2014) conducted a field trial to study the *in vivo* efficacy of some antibiotics against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* and found that among the different antibiotics tested a combination of streptocycline (300 ppm) + ampiclox (500 ppm) was more effective in reducing the disease incidence and severity followed by streptocycline (500 ppm) + COC (0.3%).

Bhattiprolu (2015) evaluated the efficacy of kresoxim methyl against foliar diseases of cotton and found that among the 3 doses of kresoxim methyl, kresoxim methyl at 500 ml/ha gave better disease control of 51.02%. The kresoxim methyl and propiconazole significantly increased the yield to the tune of 59.66 and 56.99 per cent, respectively.

Haq *et al.* (2015) worked on management of newly emerging bacterial seed and boll rot of cotton with antibiotics, homeopathic products and plant extracts. In field conditions, treatments proved effective *in vitro* were assessed on four cotton varieties. The result revealed that streptomycin sulphate was found the most significant with respect to *Tuberculinum aviaire* and *Datura metel*, controlling 45.81% disease severity.

Ingole *et al.* (2015) worked on chemical management of bacterial blight of cotton. The results revealed that the minimum percent disease intensity (11.30%) was recorded in treatment of 5 sprays of copper oxychloride (0.3%) + streptocycline (100 ppm) were effective in reduction of 50.80 percent disease.

Ashish *et al.* (2016) studied the efficacy of agro-chemicals on severity of bacterial blight of pomegranate. Three sprays of the agro-chemicals were done at 15 days interval starting from end June to end July on Mridula variety of pomegranate. Among the various treatments, Blitox (0.3%) + Streptocycline (250 ppm) proved most effective in reducing per cent disease index, per cent fruit cracking and providing maximum disease control. Quality parameters viz.,

TSS, acidity, weight, pulp colour, juice weight etc. were also studied. Maximum TSS, fruit weight, juice weight, pulp weight, 100 grain weight and total grain weight were observed in Blitox (0.3%) + Streptocycline (250 ppm) followed by Kocide (0.25%) + Streptocycline (250 ppm) sprayed fruits.

Sajid *et al.* (2017) conducted green house experiment whose results revealed that the best the combination of Flare and *N. tabacum* had the lowest disease incidence (32.27%) at all the tested concentration. Same results were obtained in field experiment, where the lowest disease incidence (40.41%) was recorded when the Flare and *N. tabacum* were applied in combination, although it was higher than green house.

11.5 Host range of *Xanthomonas axanopodis* pv. *malvacearum*.

Wernham *et al.* (1948) found that *X. campestris* pv. *malvacearum* was host specific to cotton and can infect *Brassica oleracea* var. *capitata*, *Cucurbita maxima*, *Hedera helix*, *Papverrh oas*, *Phaseolus vulgaris*, *Glycine max*, *Prunus persica*, *Saccharum officinarum*, *Hordeum vulgare*, *Triticum aestivum* and *Lycopersicon esculentum*. *Thurbiriathes pesiodies*, *Eriodendron anfructuosum*, *Jotropha curcas*, *Lochnera pusilla*, *Thespesia populnea*, *Thespesia lambas* and *Phaseolus vulgaris* and are reported to be collateral hosts of *X. campestris* pv. *malvacearum* (verma, 1986; Srinivasan 1994; singh 1998). *X. campestris* pv. *malvacearum* was also isolated from *Cieba pentandra* (verma, 1986). Verma (1986) also observed that in needle preeck inoculation *Albutilon indicum*, *Aristolochia bracteata*, *Hibiscus rosasinensis*.

Valerie *et al.* (1988) reported that *Xanthomonas campestris* pv. *translucens* is the causal agent of bacterial leaf streak of cereal grains and grasses, and individual strains within the pathovar differ in their host range among the cereals. Co-inoculation of a wide-host-range and a narrow-host-range strain resulted in the wide-host-range reaction. Transposon and chemical mutagenesis of the wide-host-range strain *X. campestris* pv. *translucence* 4, pathogenic on Barley, Wheat, Rye, and Triticale, resulted in variants with reduced host range. When pathogenicity was inactivated independently for

Barley, Wheat, Triticale, and Rye, wild-type symptoms were retained on the other members in the host range.

Gholve *et al.* (2005) evaluated the suitability of 11 field crops, 10 plantation crop tree and 42 weeds as a host to *X. axonopodis* pv. *malvacearum* and get infected by the pathogen among the weed *Euphorbia geniculate*, *Acalypha indica*, *Corchorus acutangulus*, *phaseolus* spp. and *Cassia tora* developed diseased symptoms and thus, were considered host of *X. axonopodis* pv. *malvacearum*.



Materials and Methods



CHAPTER-III

MATERIALS AND METHODS

In the present investigations on bacterial blight of cotton (*Xanthomonas axonopodis* pv. *malvacearum*), various experiments were conducted at the Department of Plant Pathology, College of Agriculture, Vasant Rao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani and Cotton Research Station, Nanded during 2017-2018 to fulfill the objectives defined. The details of the materials used and methods followed for various experiments are described here in the following paragraphs.

3.1. Materials

3.1.1. Site of experiment

Land resource for conducting field experiment was utilized from Cotton Research station, Nanded. All the laboratory experiments were conducted in the laboratory of Department of Plant Pathology, VNMKV, Parbhani.

3.1.2. Culture media

Nutrient Agar medium and Yeast Glucose Chalk Agar (YGCA) medium were used as basal medium for isolation and sub culturing of *Xanthomonas axonopodis* pv. *malvacearum*. For mass multiplication of the bacterium, Nutrient broth was used and the pure culture of *Xanthomonas axonopodis* pv. *malvacearum* was maintained on Yeast Glucose Chalk Agar (YGCA) medium. The readymade (Hi-media) synthetic media and ingredients of non-synthetic media were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.3. Chemicals

Standard chemicals, reagents, bactericides, culture media etc. required for the experimentation were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.4. Glasswares

The common glasswares (J-SIL make) viz., Petri-dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rods, beakers, funnel, pipette, airtight plastic polypropylene tubes etc. were obtained from the Department of Plant Pathology, College of Agriculture, Parbhani.

3.1.5. Equipments

The laboratory equipments viz., Autoclave, Hot air oven, Laminar airflow Cabinet, BOD Incubator, Refrigerator, Binocular Research Microscope, Electronic balance etc. available at the Department of Plant Pathology, College of Agriculture, Parbhani were utilized as and when required.

3.1.6. Seeds

Cotton seed variety Jaadoo Bt was obtained from the Cotton Research Station, Nanded V.N.M.K.V., Parbhani

3.1.7. Antibacterial chemicals

The following 3 antibiotics and 4 antibacterial fungicides were used for *in vitro* experiments.

Sr.No .	Common name	Trade Name	Manufacture
1.	Streptomycin Sulphate	Streptocycline	Hindustan Antibiotics Ltd., Mumbai
2.	2-bromo-2-nitropropane-1,3-diol	Bactrinashak	Indofil Chemicals Company, Delhi
3.	Copper oxychloride	Blitox	Syngenta Ltd., Mumbai
4.	Copper hydroxide	Kocide	Green crop International Ltd., Pune

3.1.8. Miscellaneous

Earthen pots (30 cm dia.), plant protection appliances, inoculation needle, forceps, blotter paper, paper bags, polythene bags, spirit lamp, mercuric chloride, labels, scales, sand, soil, FYM, screen house etc. were used during the course of present investigation.

3.2. Methodology

3.2.1. Disease Survey

A roving disease survey of the incidence and severity of bacterial blight disease was undertaken in Parbhani and Nanded districts of Marathwada region. Total nine fields were inspected, out of which 1 to 4 were in Parbhani and 5 to 9 in Nanded district.

In Parbhani district Jintur, Gangakhed and Pathri and in Nanded district Nanded, Loha Kandhar tehsils were inspected for recording bacterial blight disease incidence and its severity on cotton crop.

Incidence of bacterial blight disease was examined as severe, moderate, trace and free on the basis of percentage of severity of disease. Similarly, the per cent disease incidence of bacterial blight was calculated by using formula

$$\text{Percent disease incidence} = \frac{\text{No. of plants infected}}{\text{Total no. of plants examined}} \times 100$$

Percent disease intensity

Five leaves each at bottom, middle and top were observed and scored using the 0 - 4 scale prescribed by Sheo Raj (1988) as given below:

Disease Rating Scale

Grade	Per cent of leaf area infected	Reaction
0	Completely free from foliar diseases	Immune
1	1 – 10% infection	Highly resistant
2	11 – 20% infection	Moderately resistant
3	21 – 40% infection	Moderately susceptible
4	>40% infection	Highly susceptible

The percentage disease intensity were recorded on experimental plot and calculated by using formula,

$$\text{Per cent disease intensity} = \frac{\text{Sum of all disease rating scale}}{\text{No. of rating} \times \text{maximum disease grade}} \times 100$$

3.2.2. Cleaning and sterilization of materials and reagents

All the glasswares and plastic-wares were soaked overnight in potassium dichromate sulfuric acid solution. After 24 hours, these were washed thoroughly in running tap water and rinsed with distilled water. The plastic-wares were air-dried and the glasswares were sterilized in hot air oven at 160-180° C for about 90 minutes.

Isolations, inoculations and various *in vitro* experiments were conducted under aseptic condition of laminar air flow cabinet. Before working under the hood, the working surface was uniformly sterilized using 70 per cent alcohol. The blades, forceps, inoculation loop etc. were sterilized by incineration on the flame of the spirit lamp.

3.2.2. Isolation of the pathogen

The diseased specimens of bacterial blight of cotton collected from the experimental plots of Cotton Research Station, Nanded, V.N.M.K.V., Parbhani were subjected to isolations on selective synthetic media. The leaves and bolls were washed and pieces of infected tissue were cut aseptically from the edge of typical spots with a little portion of healthy tissue. These tissues were surface disinfected in Petri plates containing 95 per cent alcohol for few seconds and immediately placed on blotting paper for the removal of excess alcohol. After this it was again disinfected in mercuric chloride (HgCl₂) solution for 30 sec and subsequently washed in sterile distilled water for 3-4 times to remove the traces of mercuric chloride. The disinfected pieces were transferred by means of sterile forceps to a drop of sterile water on a sterile glass slide and teased by means of sharp sterile razor blade.

The water drop from slide was taken with a bacterial inoculation loop and streaked on a petri plate containing NA media and YGCA media under aseptic condition. These plates were incubated at $27 \pm 1^{\circ}\text{C}$ in BOD incubator for 72 hours. After incubation the plates were observed for development of the colonies of *Xanthomonas axonopodis* pv. *malvacearum*.

3.2.3. Purification of the pathogen

The typical bacterial colonies of *Xanthomonas axonopodis* pv. *malvacearum* were picked up by sterilized inoculation loop, streaked separately on YGCA medium in sterilized Petri plates and incubated at 27 ± 1 °C for 48 hours. The observations were made for the development of well separated typical yellow colonies. The pure colonies were further transferred on the slant containing Yeast Glucose Chalk Agar (YGCA) medium. All the slants were kept at 4 °C in refrigerator as stock culture for further studies.

3.2.4. Identification of the pathogen

Identification of the pathogen causing bacterial blight of cotton was determined by conducting studies on its morphological, cultural, biochemical and physiological features of the pathogen as per standard microbiological procedures.

3.2.4.1. Morphological characters

The morphological characteristics of the pathogen such as cell shape, gram reaction, staining characters were studied as per the standard procedures described by Bradbury (1984); Schaad (1988).

a) Shape of bacterial cell

The shape of bacterial cell was studied by negative staining using 10 per cent Nigrosin solution. A loopful of bacterial suspension was mixed with an equal amount of the Nigrosin solution on the glass slide and spread evenly on a microscope slide. The smear was allowed to air dry and examined under the microscope.

b) Gram staining reaction

A loop full of the bacterium suspension was smeared on clean glass slide, air fixed by gentle heating on flame of the spirit lamp. The smear was flooded with aqueous Crystal violet solution (0.5%) for 30 seconds and then washed with running tap water for a minute. This stained smear was later flooded with Gram iodine solution for one minute and rinsed in tap water. Later

the smear was decolorized with 95% of ethanol until colour runoff, washed with water and treated with Safranin as counter stain for about 10 seconds, washed with water, air /blot dried and observed under research microscope (make : Olympus) at 100X using oil immersion technique.

3.2.5. Pathogenicity test

The virulence and pathogenic nature of the isolated *Xanthomonas axonopodis* pv. *malvacearum* bacteria was proved by spraying the bacterial suspension on the cotton plant. Five seeds of the Bt cotton variety Jaadoo BG-II were sown in each pot filled with mixture of soil and FYM (2:1 proportion) and immediately watered with sterilized distilled water. Two weeks after germination, only four seedlings were maintained in each pot. When the plants were 4-6 weeks old, a bacterial suspension (10^8 cfu/ml) was prepared as an inoculum for pathogenicity test.

The underside of the leaf surface was sprayed with water and dusted with carborendum powder. Further, these leaves were smeared with bacterial suspension by means of sterile cotton swab. Simultaneously, one of the pot were kept untreated control.

3.2.7. In vitro evaluation of chemicals

Antibiotics each at three different concentrations were evaluated for their sensitivity against the growth of *Xanthomonas axonopodis* pv. *malvacearum* by inhibition zone assay method. The bacterium were multiplied by inoculating the culture in NA broth. The bacterial suspension was then seeded to the NA medium.

Experimental details

Design	: CRD
Treatments	: 12
Replication	: 3

Treatments	Chemical name	Concentration
T ₁	Streptocycline	250ppm.
T ₂	Streptocycline	500ppm
T ₃	2 Bromo 2 Nitro propane 1,3,diol	250ppm
T ₄	2 Bromo 2 Nitro propane 1,3,diol	500ppm
T ₅	Streptocycline 250ppm+ Copper oxychloride 0.25%	250ppm+0.25%
T ₆	Streptocycline 500ppm+ Copper oxychloride 0.25%	500ppm+0.25%
T ₇	2Bromo250ppm+copper oxychloride 0.25%	250ppm+0.25%
T ₈	2Bromo500ppm+copper oxychloride 0.25%	500ppm+0.25%
T ₉	Strepto250ppm.+copper hydroxide 0.1%	250ppm+0.1%
T ₁₀	Strepto500ppm.+copper hydroxide 0.1%	500ppm+0.1%
T ₁₁	2 Bromo +copper hydroxide 0.1%	250ppm+0.1%
T ₁₂	Control	

The antibiotic solutions were prepared at different concentrations. The filter paper discs measuring 5 mm diameter were soaked in respective antibiotic solutions and it then were transferred to the medium of plates.

The inoculated plates were kept in the refrigerator at 5⁰C to allow the diffusion of chemical in to the medium. The plates were then incubated at 27⁰C and observed for the production of inhibition.

Bio efficacy of these antibiotics and fungicides were evaluated at different concentrations mentioned in the treatment details.

Observations regarding the inhibition zone by antibiotics were recorded at 48 – 72 hours after inoculation.

The inhibition zones were calculated by the formula given by Vincent (1927).

$$\text{Percent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition of growth

C = Growth (mm) of test bacteria in untreated control plates

T = Growth (mm) of test bacteria in treated plates

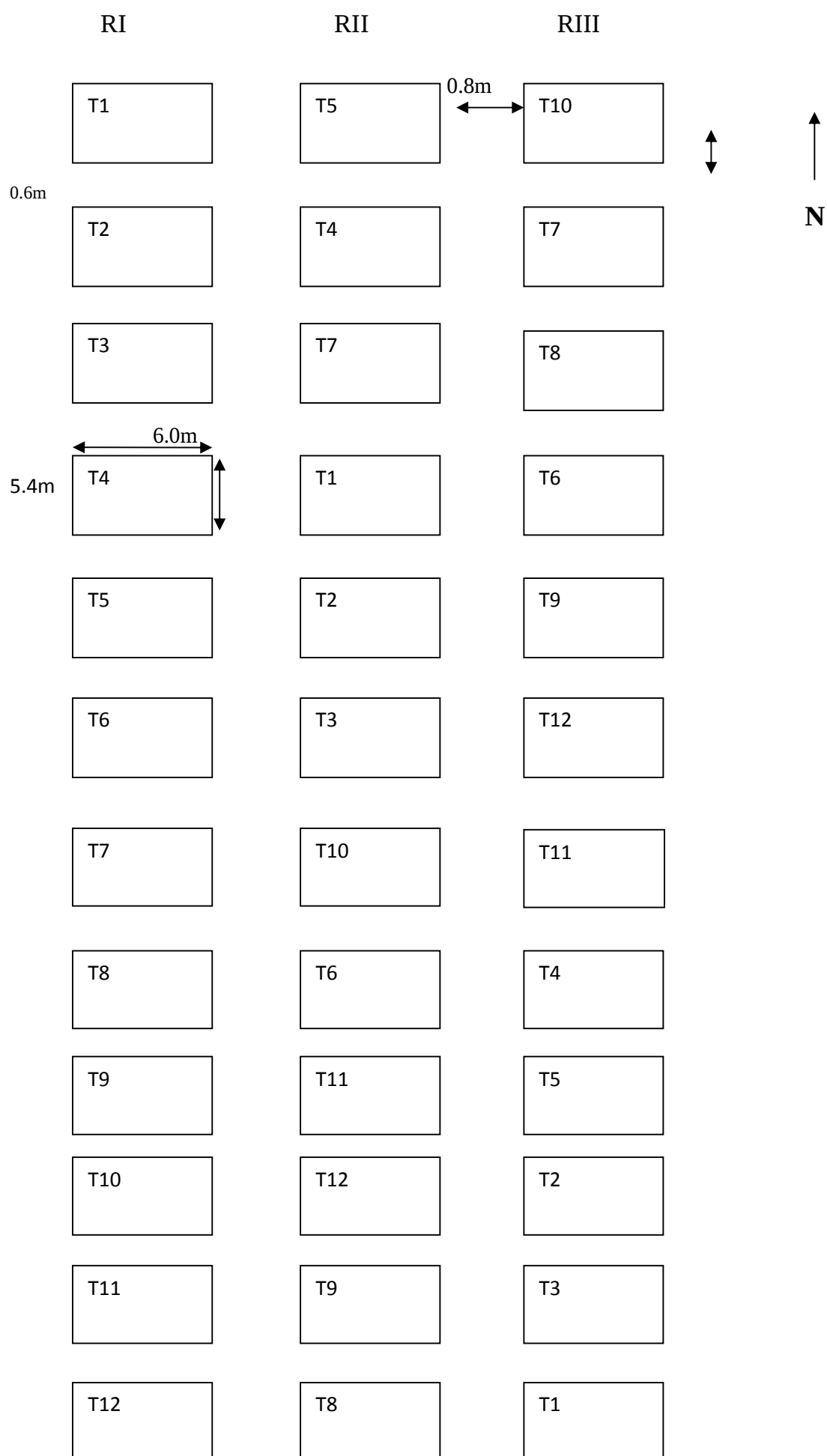


Fig. 1. Field layout of trial on bacterial blight of Bt cotton (var. Jaadoo)

3.2.8. *In vivo* evaluation of chemicals

Field experiment on management of bacterial blight of cotton was conducted at Cotton Research Station, Nanded. Experiment was laid out in Randomized Block Design with 12 treatments replicated thrice. Unsprayed plants was served as control and three sprays were given as per the schedule given in treatment details.

Experimental details for Bt cotton

Design	: RBD
Treatments	: 12
Replication	: 3
Plot size	: 6 m x 5.4 m
Spacing	: 120 x 45 cm
Spacing between plots	: 0.6 m
Spacing between replication	: 0.8 m
Variety	: Jaadoo Bt (susceptible variety)
Season	: <i>Kharif</i>
Location	: Cotton Research Station, Nanded
Date of sowing	: 28/06/2017

Treatment Details

Treatments	Chemical name	Concentration
T ₁	Streptocycline	250ppm.
T ₂	Streptocycline	500ppm
T ₃	2 Bromo 2 Nitro propane 1,3,diol	250ppm
T ₄	2 Bromo 2 Nitro propane 1,3,diol	500ppm
T ₅	Streptocycline 250 ppm+ Copper oxychloride 0.25%	250ppm+0.25%
T ₆	Streptocycline 500 ppm+ Copper oxychloride 0.25%	500ppm+0.25%
T ₇	2Bromo 250 ppm+copper oxychloride 0.25%	250ppm+0.25%
T ₈	2Bromo 500ppm+copper oxychloride 0.25%	500ppm+0.25%
T ₉	Strepto 250ppm.+copper	250ppm+0.1%

	hydroxide 0.1%	
T ₁₀	Strepto 500 ppm. + copper hydroxide 0.1%	500 ppm + 0.1%
T ₁₁	2 Bromo + copper hydroxide 0.1%	250 ppm + 0.1%
T ₁₂	Control	

Spraying schedule

1. First spray immediately after disease appearance.
2. Subsequent spray at 15 days after first spray.
3. Immediately after receipt of rains during flowering / seed setting.

Observations on disease incidence and severity were recorded at 15 days interval after each spray.

Host range of *Xanthomonas axanopodis* pv. *malvacearum*

Ten field crops and seven plantation crops were spray inoculated with the suspension of *X. axanopodis* pv. *malvacearum* culture in water (approximately 10⁶ cells/ ml) in active growing stage of crop.

Observations regarding appearance of symptoms were recorded by frequent visits. The details of field crops inoculated age of the crop and date of inoculation are depicted in table.

Details of field crops inoculated with *Xanthomonas axanopodis* pv. *malvacearum*

Sr.No.	Name of the crop	Age of the crop (weeks)	Date of Inoculation
1	Soybean (<i>Glycine max</i>)	6	25 July 17
2	Pigeon pea (<i>Cajanus cajan</i>)	8	5 Sept. 17
3	Green gram (<i>Vigna radiata</i>)	6	25 July 17
4	Black gram (<i>Vigna sinensis</i>)	5	20 July 17
5	Castor (<i>Ricinus communis</i>)	7	9 Sept 17
6	Maize (<i>Zea mays</i>)	8	17 Aug. 17
7	Wheat (<i>Triticum aestivum</i>)	8	9 Jan. 18
8	Safflower (<i>Carthamus tinctorius</i>)	9	9 Jan 18
9	Gram (<i>Cicer arietinum</i>)	7	5 Dec 17
10	Sorghum (<i>Sorghum vulgare</i>)	6	10 Dec.17

Plantation crop and tree crop hosts

Commonly available plantation crops like mango (*Mangifera indica*), guava (*Psidium guajava*), tamarind (*Tamarindu indica*), pomegranate (*Punica granatum*), and tree hosts like Baniyan tree (*Haeroglyph ousbaniyan*), Babool (*Acasis arabica*), Ashoka tree (*Haerogly phousbaniyan*) were spray inoculated with suspension of *Xanthomonas axanopodis* pv. *malvacearum* as per procedure. Observations regarding appearance of symptoms were made by frequent visits.

The infection was confirmed by reisolation and establishing the identity of the pathogen.

3.2.9. Statistical analysis

The data obtained in all the experiments were statistically analyzed. The percentage values were transformed into arcsine values. The standard error (S.E.) and critical difference (C.D.) at level $P = 0.01$ were worked out and results obtained were compared statistically. All the statistical analysis was done using VNMKV-STAT statistical programmer at Central Computer Laboratory, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.



Results and Discussion



CHAPTER-IV

RESULTS AND DISCUSSIONS

Present studies on the bacterial blight [*Xanthomonas axonopodis* pv. *malvacearum* of cotton (*Gossypium arboreum* and *Gossypium hirsutum*) were undertaken during 2017 on the aspects viz., survey, isolation, Purification, identification, morphology, cultural characters, pathogenicity, symptomatology, *in vitro* efficacy of antibiotics, antibacterial fungicides, *in vivo* efficacy of antibiotics, antibacterial fungicides and host range. The results obtained on all these aspects are narrated as follows under various sub-heads.

4.1 Survey

A roving survey was carried out during Sept-Oct, 2017 at different locations of Nanded and Parbhani districts for recording the percent diseases severity and incidence of bacterial blight of cotton in field. The plant showing typical symptom of bacterial blight of cotton disease was conformed through ooze test. Total twenty six cotton field were observed in Parbhani and Nanded districts of Marathwada region. (Table 1, Fig.2, 3 and PLATE I (A, B,))

Table 1. Survey of bacterial blight severity of cotton in different districts of Maharashtra region.

Sr. No .	District	No. of field surveyed	Variety	Disease Severity				
				Severe	Moderate	Trace	Free	Total
1.	Parbhani	14	Mallika Bg-II	3	1	1	-	5
			Vitthal	2	2	-	-	4
			Ajeet-155	3	2	-	-	5
			Total	8	5	1		14
2.	Nanded	12	Mallika Bg-II	2	2	1	-	5
			Vitthal	2	1	1	-	4
			Ajeet-155	2	1			3
			Total	6	4	2	-	12

Severe - > 50%

Moderate- >10% to <50 %

Trace- < 10%

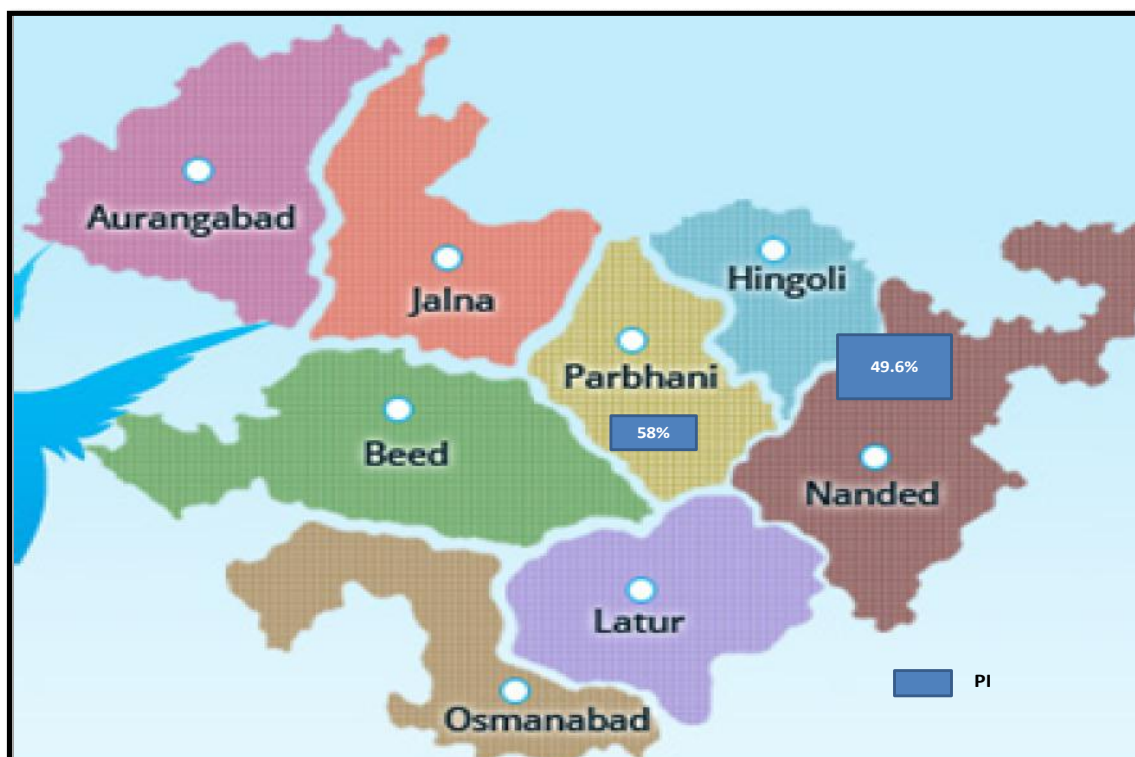


Fig. 2 : Map of Marathwada region showing district wise average bacterial blight incidence (PI) during 2017



Survey on Parbhani districts



Survey on Nanded districts

Plate 1. Survey on Parbhani and Nanded districts.

4.1.1 Occurrence of bacterial blight of cotton incidence in Parbhani and Nanded districts

Data pertaining to percentage disease incidence of bacterial blight of cotton in Parbhani and Nanded districts are presented in table 1 and figure.1

During *Kharif* 2017, survey of bacterial blight severity on cotton was undertaken in Parbhani and Nanded districts of Marathwada region. The highest disease severity was recorded on Var, Mallika and Ajeet in Parbhani district followed by moderate intensity on Vitthal in Nanded District. The variety Mallika Bt was highly susceptible to disease in both the districts followed by Ajeet-155 and Vitthal.

4.1.2. Per cent disease incidence of bacterial blight of cotton in Nanded and Parbhani districts

The data presented in table-2 revealed that the incidence of bacterial blight of cotton in the districts surveyed ranged from 49.6% to 58% in Parbhani and Nanded district, respectively.

Govindappa *et al.* (2008) conducted survey in areas of Dharwad Haveri, Belgaum, Bagalkot, Bellery, Gahaeg, Raichur and Gulbarga districts of North Karnataka to assess the incidence of bacterial blight of cotton on farmer field and Agriculture Research Station. Bacterial blight was only in Dharwad districts of ARS Dharwad farm and MARS Dharwad recorded 25 % of 5% bacterial blight incidence. Haveri district recorded 10% bacterial blight incidence.

Jagtap *et al.* (2012) conducted extensive roving survey in different districts of Marathwada region of Maharashtra state to isolate the pathogen associated with the bacterial blight disease of cotton. All 76 cotton fields were surveyed and average disease incidence (PI) to the tune of 51.12 per cent was observed. Highest disease incidence noticed in Parbhani district (67%) followed by Hingoli (63%), Nanded (58%) and Latur (54%). The lowest disease incidence noticed in Jalna district (36%). Highest disease severity

noticed in Parbhani district followed by Hingoli, Latur, Nanded and Aurangabad.

Patole *et.al.* (2016) Cotton, “king of fibres” enjoys a pre-eminent status among all cash crops in the country, being the principal raw material for a flourishing textile industry. Among the various diseases occurring on cotton, the foliar disease bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum* is gaining more importance in recent years because of their increasing incidence. These have been known to occur on all the various cultivated and wild species of cotton in Maharashtra, since many years, in an epiphytotic form on commercially grown varieties, which leads to severe defoliation and substantial yield losses. Among the 221 cultivated genotypes screened for resistance against bacterial leaf blight disease under field conditions, 80 genotypes showed immune reaction. Further, 69 genotypes were resistant, 13 genotypes were moderately resistant, The 19 entries showed the moderately susceptible reaction and 40 entries showed the susceptible reaction to the bacterial blight disease.

Prashant *et al.* (2017) conducted a roving survey on cultivars' field during the crop season and a fix point survey on cotton was conducted periodically at Research farm. On Research farm, periodical observations were recorded on G. Cot. Hy. 12 which revealed that bacterial blight appeared during the 2nd week of July (2.0 %) and then gradually developed and reached at its peak (23.5 % PDI) during the 3rd week of September and then declined, but prevailed up to 1st week of December. Observations were also recorded on other cultivars. The susceptible cultivars viz., LRA 5166 showed bacterial blight intensity to the tune of 2.5 to 23.0 per cent PDI moreover, non Bt cotton was more susceptible to the bacterial blight disease

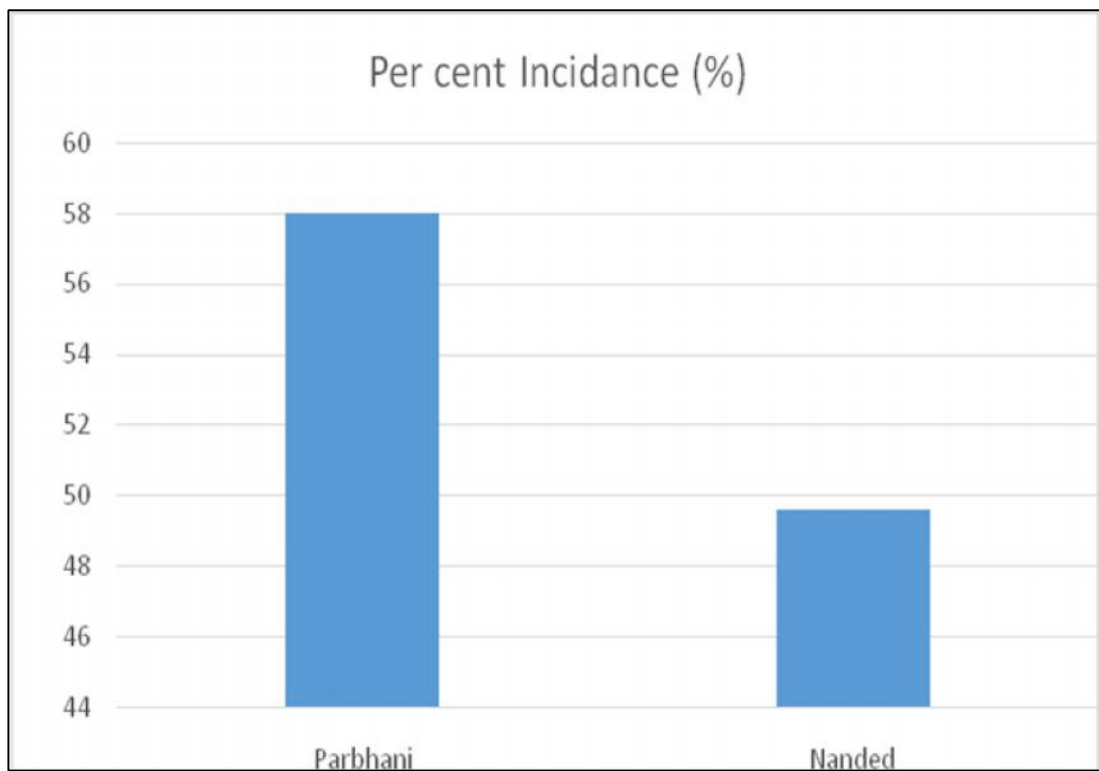


Fig. 3 : Per cent disease incidence of bacterial blight of cotton in Parbhani and Nanded districts of marathwada region

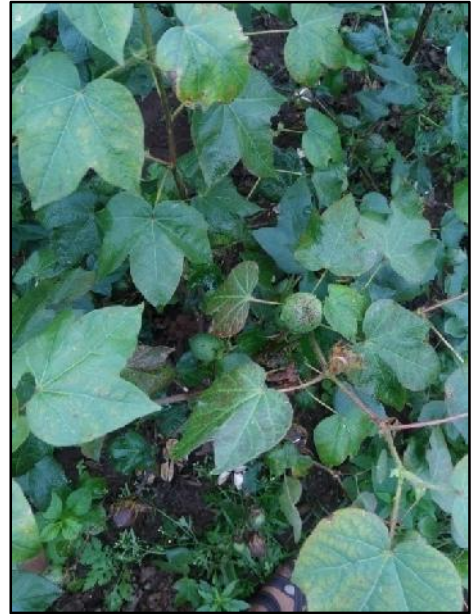
Table 2. Survey of bacterial blight incidence of cotton in different districts of Maharashtra region.

Sr. No.	Districts	Total number of field Surveyed	Total number of plant examined	Infected plant with bacterial blight	Per cent disease incidence (%)
1	Parbhani	14	360	210	58
2	Nanded	12	250	124	49.6
				Total	53.8

4.2. Symptomatology

In field, first symptoms of bacterial blight disease were observed at 30 days after sowing of cotton crop. The symptoms of the disease were first noticed as initial minute lesions, which were water soaked and scattered on the under surface of the young leaves. The lesions gradually increased in size (upto 5mm dia), first turned brown and then black and formed angular dead areas with reddish border bounded by veinlets. The lesions extended along of the veins and observed vein blight symptoms PLATE II (A,B,C,)

Allen (2014) described the symptoms expression on individual plant parts. Symptoms on leaves – lesions on leaves appear as small, angular spots that in some cases can congregate around the veins. The angular nature of the spots are produced by the capillary veins limiting the spread of the bacterium within leaf tissues. By flipping the leaf over and observing the underside of the leaf the water-soaking around the spot will develop a greasy appearance, especially in the presence of morning dew. He added that if having trouble determining bacterial blight from other diseases (or even herbicide injury) look for two specific characteristics: 1) a systemic vein infection, generally in the mid to upper canopy and 2) the appearance of extreme water-soaking around lesions that may congregate on leaf tissues. On bracts – lesions on bracts look more elongated than on leaves and are generally brown to maroon in color.



A) Angular leaf spot of cotton



B) Boll rot

**Plate 2. Typical symptoms of *X. axonopodis* pv. *malvacearum* on Bt cotton
(var. Jaadoo BG-II)**

4.3. Isolation of *Xanthomonas axonopodis* pv. *malvacearum* from infected leaf

The pathogen was isolated from the diseased cotton leaves by tissue isolation method on nutrient agar medium. Yellow glistening colonies with smooth, profuse and mucoid growth began to appear after 48 to 96 hrs of incubation at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The purified *Xanthomonas* bacteria were maintained at 4°C .

4.4. Purification of bacterial culture

Well separated colonies *Xanthomonas axonopodis* pv. *malvacearum* on YGCA medium and NA medium isolated from the infected leaves were further purified by streaking on the surface of YGCA medium (PLATE III). The culture was maintained on Yeast Glucose Chalk Agar (YGCA) slants (PLATE III (A and B) at 5°C . The bacterial colonies on YGCA medium were yellow, mucoid, convex, glistening and irregular to round in shape (PLATE III (A). Nutrient broth was used for the mass multiplication of the bacterium (PLATE III).

4.5. Identification of the pathogen

Identification of the isolate obtained from diseases sample of cotton was done by conducting studies on morphological, cultural, biochemical and physiological features of the pathogen based on the standard microbiological procedures. The test pathogen under study were identified and confirmed as *Xanthomonas axonopodis* pv. *malvacearum*, the cause of bacterial blight of cotton.

4.5.1. Morphological Characters

The results on morphological studies are presented in the Table 3, PLATE IV. The results indicated that the bacterium is rod shaped with rounded ends, cells appeared single, gram negative having single polar flagellum.

The result obtained in the present study on morphological characters were in agreement with the reports of earlier workers. (Bradbury, 1984);



Plate 3. Isolation of *Xanthomonas axonopodis* pv. *malvacearum* of cotton.

McGuire *et al.*, (1986); Carrillo *et al.*, (2001); Bobosha (2003); Dezordi *et al.*, (2009); Hamid *et al.*, (2012); Raghuwanshi *et al.*, (2013) and Haq *et al.*, (2015).

Table 3. Morphological characteristics of *Xanthomonas axonopodis* pv. *malvacearum*

Sr. No.	Characters	Isolates
1.	Shape	Rod
2.	Occurrence / arrangement	Single
3.	Flagellation	Monotrichous
4.	Gram reaction	Negative

4.5.2. Cultural characters

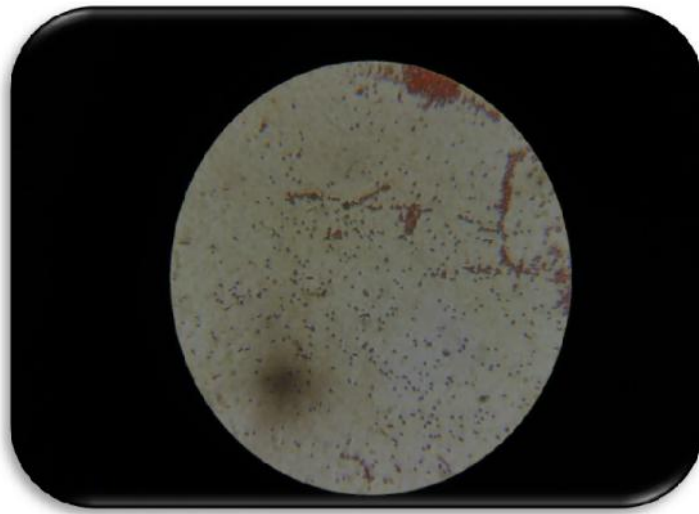
Colony characteristics of the pathogen differed with respect to media and the results are presented in the Table 4, PLATE V. The Convex, smooth, shiny, flat glistening, light yellow to yellow coloured colonies were observed on Nutrient Agar. Colonies were circular to irregular, mucoid, flattened and yellow coloured on YGCA medium.

Similar observations were also reported by Malavolta *et al.* (1999), El-Sadek *et al.* (2001), Dhutraj and Gokhale (2007) and Opara *et al.* (2009).

Table 4. Growth and cultural characters of *Xanthomonas axonopodis* pv. *malvacearum* on different agar media

Sr. No.	Medium	Growth characters after 48 h of incubation
1	Nutrient agar	Initially colonies were filiform and light yellow then becoming waxy yellow, growth was abundant
2	YGCA	Growth fairly good, filiform slightly raised with entire margin, glistening, yellowish, slightly turn brown

The results growth of bacterium on different agar plates are presented in the Table 5, PLATE V. The growth was fairly good, filiform, slightly raised, light yellow colonies on Nutrient Agar medium. Growth was abundant, filiform, glistening yellow colonies on YGCA medium.



Gram staining

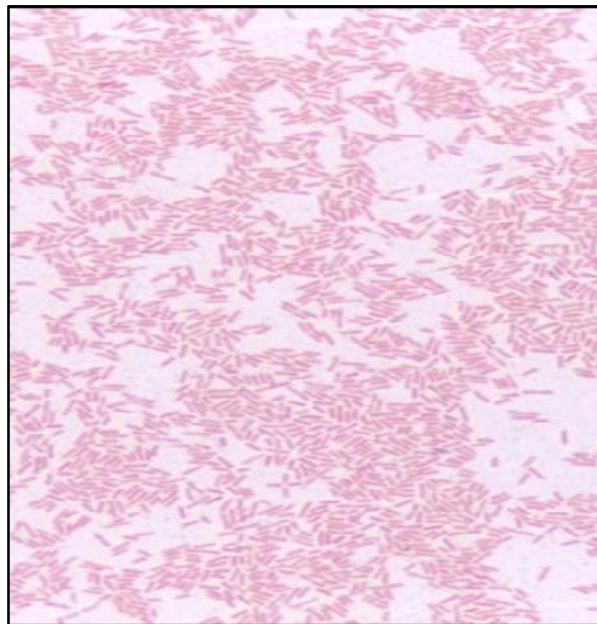


Plate 4. Morphological characteristics of *Xanthomonas axonopodis* pv. *malvacearum*



NA



YGCA



NA



YGCA



NA



YGCA

Plate 5. Growth of *X. axonopodis* pv. *malvacearum* on different media.

Table 5. Growth and cultural characters of *Xanthomonas axonopodis* pv. *malvacearum* on different agar plates.

Sr. No.	Medium	Growth characters after 48 hrs of incubation
1	Nutrient agar	Initially colonies were filiform and light yellow then becoming waxy yellow, growth was abundant
2	YGCA	Colonies were filiform, slight brown colours, growth was abundant

The results of growth characters of bacterium on different broth are presented in the Table 6, PLATE V .The medium was slightly turbid on Nutrient broth. Turbid growth throughout the medium was observed on YGCA broth.

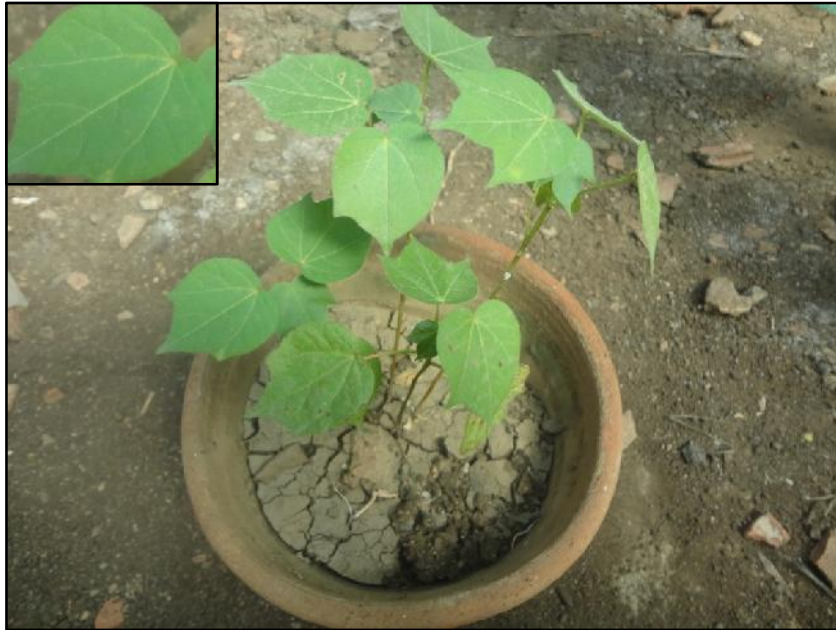
Table 6. Growth and cultural characters of *Xanthomonas axonopodis* pv. *malvacearum* in different broths.

Sr. No.	Name of broth	Surface growth	Turbidity	Amount of growth
1	Nutrient broth	Slight growth, slightly fluorescent	Light cloudy	Poor
2	YGCA	Pellicle	Light cloudy	Moderate

Abundant growth, filiform nature, glistening, butyrous, pale yellow colonies observed on nutrient broth medium *in vitro*. Also filiform, glistening, yellowish, fairly good growth observed on chalk agar medium *in vitro* study.

4.6. Pathogenicity

Pathogenicity of *Xanthomonas axonopodis* pv. *malvacearum* was proved by spray inoculation of the bacterial suspension on Bt cotton. Jaadoo, BG-II, (PLATE VI) (A) in pots at ten to twelve leaf stage, after predisposition to humid condition for 24 h. The leaves were inoculated with the homogenized culture of *Xanthomonas axonopodis* pv. *malvacearum*. Prior to automization of the culture on leaf, the leaves were slightly injured with carborendum powder. Seedlings were then retained under humid condition for 24 h by covering the



Healthy Plant



Diseased Plant

Plate 6. Symptoms in injury with carborandum powder followed by spray inoculation.

pots with plastic bag. Pots were adequately watered. Simultaneously uninoculated plants were maintained as control.

The symptoms initiated in the form of lesions, distinctly visible and appeared within 3-4 days after inoculation. The spots initially appeared as water soaked lesions, which later turned into brown to black spots.

Re-isolation was carried out from the lesions and comparison was made with original culture to confirm the identity of the pathogen. The isolates from artificially inoculated plants yielded the bacterial colonies similar to the original ones.

Pathogenicity of *Xanthomonas axonopodis* pv. *malvacearum* causing cotton bacterial blight were proved by several workers. Akthar and Khan (1998), Vincent *et al.* (2001), Hasan *et al.* (2008), Razaghi *et al.* (2012) and Jagtap *et al.* (2012).

4.7. *In vitro* evaluation of different chemicals against *Xanthomonas axonopodis* pv. *malvacearum*

Efficacy of different chemicals *in vitro* was evaluated against the *Xanthomonas axonopodis* pv. *malvacearum*. The data so obtained are presented in (Table 7, Fig.4 and PLATE VII (A)) clearly showed that the maximum mean inhibition of colour diameter was in the treatment Streptocycline 500 ppm + Copper oxychloride 0.25% (21.25mm), Streptocycline 500 ppm. + copper hydroxide 0.1% (20.46mm), Streptocycline 500 ppm (20.23mm), 2 Bromo 500 ppm + copper oxychloride 0.25% (19.24mm), 2 Bromo 250 ppm + copper oxychloride 0.25%(18.30mm), 2 Bromo 2 Nitro propane 1,3,diol 500 ppm (17.83mm) Streptocycline 250 ppm+ Copper oxychloride 0.25% (17.46mm), Streptocycline 250 ppm + copper hydroxide 0.1% (16.39mm), Streptocycline 250 ppm (16.25mm) 2 Bromo 2 Nitro propane 1,3,diol 250ppm (15.87mm), 2 Bromo +copper hydroxide 0.1% (15.00mm)

The maximum percent inhibition of growth was found in Streptocycline 500 ppm+ Copper oxychloride 0.25% (21.25 mm and minimum percent

inhibition was found in 2 Bromo + copper hydroxide 0.1% (15.00mm) Results obtained were in accordance with Thwipa *et al.* (2016) who studied fourteen strains of antagonistic bacteria *Paenibacillus*, water extracts and oil of some plants (*Syzygium aromaticum*, *Nigella sativa*, *Trigonella*, *foenum, graecum* and blubs of *Allium sativum*) and camel urine for their efficacy in inhibiting the growth of *Xanthomonas axonopodis* pv. *malvacearum* *in vitro*.

Table 7. *In vitro* efficacy of chemicals agent against *Xanthomonas axonopodis* pv. *malvacearum*

Sr. No.	Treatment details	Conc.	Mean inhibition Zone (mm)	Growth of Pathogen (mm)	Percent inhibition
T ₁	Streptocycline	250ppm.	16.25 (9.35)	73.75	18.05
T ₂	Streptocycline	500ppm	20.23 (11.67)	69.77	22.47
T ₃	2 Bromo 2 Nitro propane 1,3,diol	250ppm	15.87 (9.13)	74.13	17.63
T ₄	2 Bromo 2 Nitro propane 1,3,diol	500ppm	17.83 (10.27)	72.17	19.81
T ₅	Streptocycline 250ppm+ Copper oxychloride 0.25%	250ppm +0.25%	17.46 (10.05)	72.54	19.40
T ₆	Streptocycline 500ppm+ Copper oxychloride 0.25%	500ppm +0.25%	21.25 (12.26)	68.75	23.61
T ₇	2Bromo250ppm+copper oxychloride 0.25%	250ppm +0.25%	18.30 (10.54)	71.70	20.33
T ₈	2Bromo500ppm+copper oxychloride 0.25%	500ppm +0.25%	19.24 (11.09)	70.76	21.37
T ₉	Strepto250ppm.+copper hydroxide0.1%	250ppm +0.1%	16.39 (9.43)	73.61	18.21
T ₁₀	Strepto500ppm.+copper hydroxide0.1%	500ppm +0.1%	20.46 (11.80)	020269.54	22.73
T ₁₁	2 Bromo +copper hydroxide 0.1%	250ppm +0.1%	15.00 (8.62)	75.00	16.66
T ₁₂	Control		00.00	90.00	00.00
SEM ±			0.42		
CD 1 %			1.24		

*Mean of three replications, Figures in parenthesis are arc sine transformed value

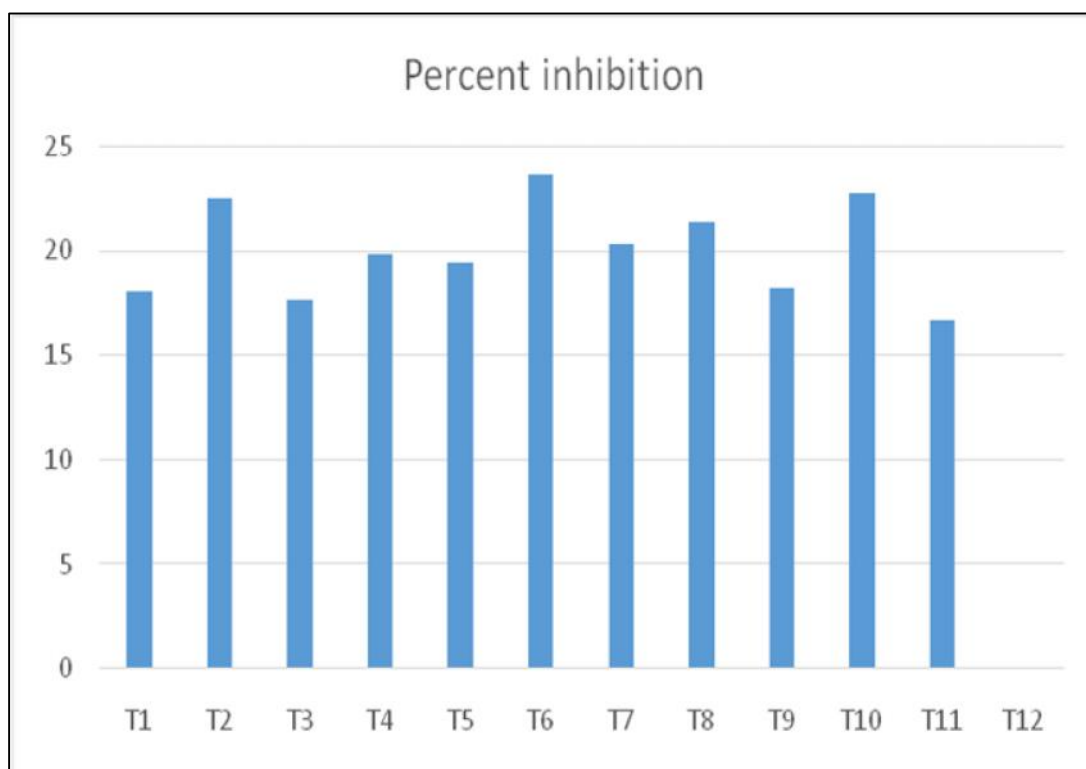


Fig. 4 : Efficacy of different chemicals against *Xanthomonas axonopodis* pv. *malvacearum*

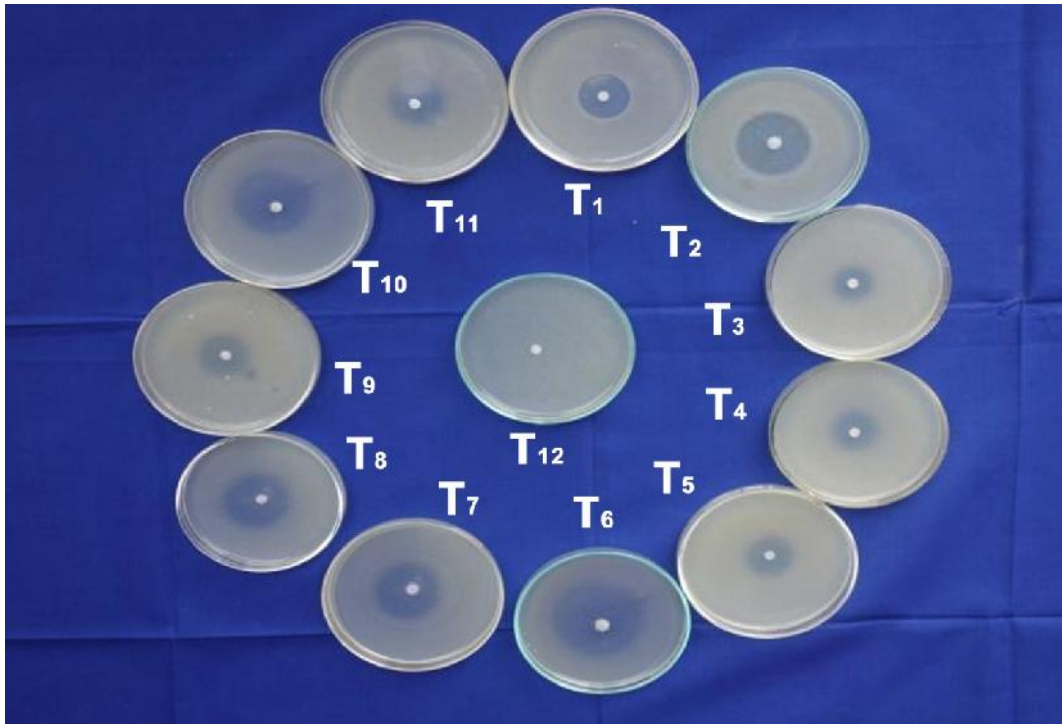


Plate 7: Efficacy of different chemicals against *Xanthomonas axonopodis* pv. *malvacearum*

T₁ : Streptocycline 250 ppm

T₂ : Streptocycline 500 ppm

T₃ : 2 Bromo 2 Nitro propane 1,3,diol 250 ppm

T₄ : 2 Bromo 2 Nitro propane 1,3,diol 500 ppm

T₅ : Streptocycline 250 ppm+ Copper oxychloride 0.25%

T₆ : Streptocycline 250 ppm+ Copper oxychloride 0.25%

T₇ : 2Bromo250ppm+copper oxychloride 0.25%

T₈ : 2Bromo250ppm+copper oxychloride 0.25%

T₉ : Strepto250ppm.+copper hydroxide 0.1%

T₁₀ : Strepto250ppm.+copper hydroxide 0.1%

T₁₁ : 2 Bromo +copper hydroxide 0.1%

T₁₂ : Control

The results are in accordance with the findings of Haq *et al.* (2015) who studied the *in vitro* efficacy of antibiotics against *Xanthomonas axonopodis* pv. *malvacearum* and found that streptomycine sulphate (1.10 cm) at 1000 ppm was the significant dose against bacterial growth followed by oxytetracycline.

Also the findings of Ehetisham-ul-haq *et al.* (2013) are same with the studies who studied *in vitro* evaluation of three chemicals against colony growth of *X. axonopodis* pv. *malvacearum*. Plant protector chemical was found effective at 600 ppm

4.8. *In vivo* evaluation of different chemicals against *Xanthomonas axonopodis* pv. *malvacearum*

A field experiment had twelve spray treatments which included 11 chemicals and one untreated control. In all 4 sprays were given at an interval of 15 days starting from disease initiation and observations on disease incidence (PI) and disease severity (PDI) were recorded.

4.8.1. Disease incidence (PI)

4.8.1.1. Per cent disease incidence of bacterial blight

Data on per cent disease incidence of bacterial blight are presented in Table 8 and Fig. 5. and PLATE VIII (A,B,C,) and results obtained were non-significant at before spray of various chemicals and the results obtained were significant at first, second, third and fourth spray.

The disease initiated at 60 days after sowing (DAS). The disease incidence was recorded before spraying of various chemicals and the per cent disease incidence ranged from 20.33% (Strepto500 ppm.+copper hydroxide 0.1%) to 26.49% (2 Bromo 2 Nitro propane 1,3,diol) over 26.49 %. Treatment T₁₀ recorded minimum per cent incidence (20.67%) and maximum was observed in control plot (26.49 %).

All the treatments recorded significantly low disease incidence over control at first, second third and fourth spray. Mean per cent disease incidence ranged from 24.02 % to 49.11%.

Among the twelve chemicals significantly lowest mean disease incidence of 24.02% was recorded in the treatment of streptocycline + copper oxychloride followed by treatment of 2 Bromo 500 ppm + copper oxychloride 0.25% (PI 25.84%). Maximum inhibition (23.61%) was recorded in the treatment of T₆ (streptocycline 500ppm + copper oxychloride 0.25 %), followed by treatment T₁₀ (22.73%) (strepto 500ppm + copper hydroxide 0.1%) showing significantly better inhibition.

There were no control of pathogen in single spray. After the first spray there was increase in per cent disease incidence to a range of 28.69 % to 44.83 %.

Table.8. *In vivo* efficacy of chemicals and agent on per cent disease incidence of *Xanthomon asaxanopodis* pv *malvacerum* of Bt cotton

Tr. No	Treatments	Conc.	PI before spray	Per cent disease incidence (PI)				Mean PI
				I	II	III	I V	
T ₁	Streptocycline	250ppm.	25.77 (14.93)	30.69 (17.87)	28.86 (16.77)	27.06 (15.70)	25.04 (14.50)	27.91 (16.21)
T ₂	Streptocycline	500ppm	23.37 (13.51)	28.69 (16.67)	28.10 (16.32)	24.40 (15.30)	24.42 (14.13)	26.40 (15.60)
T ₃	2 Bromo 2 Nitro propane 1,3,diol	250ppm	26.49 (15.36)	39.50 (23.26)	32.12 (18.73)	31.10 (18.06)	27.00 (15.66)	32.43 (18.92)
T ₄	2 Bromo 2 Nitro propane 1,3,diol	500ppm	25.98 (15.06)	34.08 (19.92)	30.06 (17.49)	26.81 (15.55)	25.47 (14.75)	29.10 (16.92)
T ₅	Streptocycline 250ppm+ Copper oxychloride 0.25%	250ppm + 0.25%	22.93 (13.25)	30.16 (17.55)	28.16 (16.35)	25.37 (14.69)	21.49 (12.40)	26.29 (15.24)
T ₆	Streptocycline 500ppm+ Copper oxychloride 0.25%	500ppm + 0.25%	24.08 (13.95)	29.36 (17.07)	24.28 (14.05)	22.07 (12.75)	20.37 (11.75)	24.02 (13.90)
T ₇	2Bromo250ppm+copper oxichloride 0.25%	250ppm + 0.25%	26.47 (15.35)	30.29 (17.63)	28.39 (16.49)	24.17 (13.98)	23.67 (13.69)	26.63 (15.44)
T ₈	2Bromo500ppm+copper oxichloride 0.25%	500ppm + 0.25%	26.16 (15.17)	30.32 (17.65)	28.51 (16.57)	23.03 (13.31)	21.52 (12.42)	25.84 (14.98)
T ₉	Strepto250ppm.+copper hydroxide 0.1%	250ppm + 0.1%	20.82 (12.04)	31.64 (18.44)	30.24 (17.60)	28.25 (16.41)	23.85 (13.79)	28.49 (16.56)
T ₁₀	Strepto500ppm.+copper hydroxide 0.1%	500ppm + 0.1%	20.33 (11.73)	33.44 (19.53)	29.39 (17.09)	25.01 (14.48)	22.36 (12.92)	27.55 (16.00)
T ₁₁	2 Bromo +copper hydroxide 0.1%	250ppm + 0.1%	24.16 (13.98)	37.69 (22.15)	30.56 (17.79)	25.19 (14.59)	22.38 (12.93)	28.95 (16.86)
T ₁₂	Control		25.96 (15.05)	44.83 (26.64)	49.71 (29.81)	50.59 (30.39)	49.11 (20.73)	49.11 (26.89)
SE ±			2.24	1.13	0.98	0.87	0.81	
CD at 5%			NS	3.33	2.89	2.57	2.37	

*Mean of three replications, Figures in parenthesis are arc sine transformed value

Significantly lowest disease incidence of 24.28 % was recorded by treatment Streptocycline 500 ppm+ Copper oxychloride 0.25% followed by treatment streptocycline (28.10%) at 15 days after second spray. Thus the treatment of streptocycline 500 ppm + Copper oxychloride 0.25 % was found to be significantly superior over rest of the treatments. All the treatments found superior as against unsprayed control 49.71 %),

The results obtained after 15 days after third spray indicated that significantly lowest disease incidence of 22.07 % was recorded by the treatment Streptocycline 500ppm+ Copper oxychloride 0.25% and the

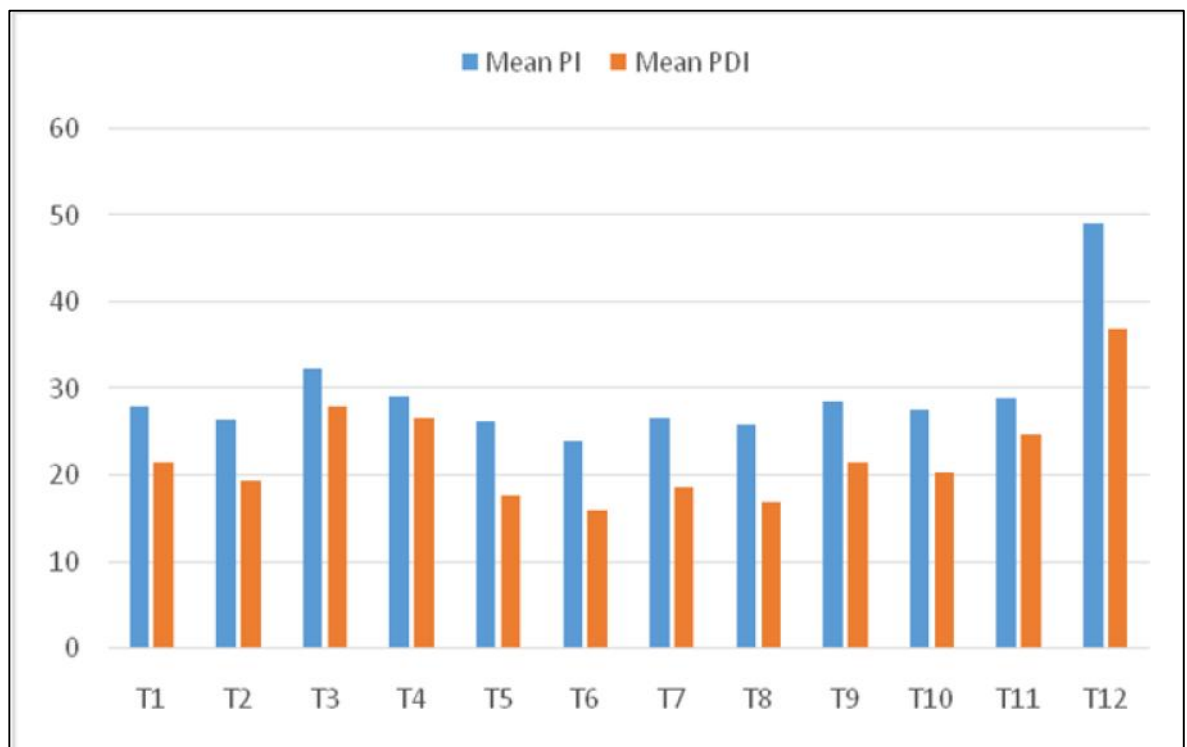


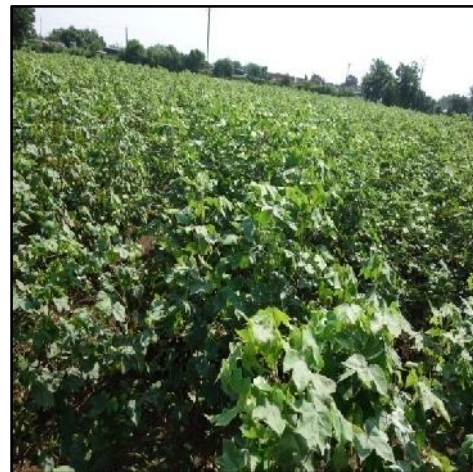
Fig. 5 : Effect of different treatments on bacterial blight incidence and intensity



Plate 8 (A). General view of experimental plot.



(B) Treatment - 6



(C) Control

Field view of the experimental plot

treatment 2 Bromo 500 ppm + copper oxichloride 0.25% (23.03%). Rest of the treatments found superior as against unsprayed control (50.59%).

The result obtained after 15 days after fourth spray indicated that significantly lowest disease incidence of 20.37 % was recorded by the treatment Streptocycline 500ppm+ Copper oxychloride 0.25% and by the treatment 2Bromo 500ppm + copper oxychloride 0.25% (21.52%). However these two treatments did not differ significantly from each other and thus were equally effective in controlling the disease. Rest of all the treatments found superior as against unsprayed control (49.11%).

4.8.2. Disease severity (PDI)

4.8.2.1. Disease severity of bacterial blight

Data on disease severity is presented in Table 9 and Fig. 5. PLATE VIII (A,B,C). The results on disease severity were significant over control at first, second, third and fourth spray. All the treatments recorded significantly low disease severity over control at first, second third and fourth spray. Mean per cent disease severity ranged from 15.97% to 36.97 %.

Disease severity before spray did not show statistically significant differences amongst the different treatments. Thus, the data recorded before spray was statistically non-significant. However the disease severity recorded before spraying of various chemical ranged from 11.97 % to 20.29 %. In plot, treatment 2 Bromo 2 Nitro propane 1,3 diol recorded low per cent incidence of 11.97% and maximum observed in treatment streptocycline 500ppm +copper hydroxide 0.1% plot of 20.29 % PDI.

Among the eleven chemicals sprayed, 15 days after the first spray significantly lowest disease severity of 19.16 % was recorded by treatment Streptocycline 500ppm+ Copper oxychloride 0.25% but was at par with treatment streptocycline + copper oxychloride 0.25% (19.42 %), 2Bromo500ppm+copper oxychloride 0.25% (19.52%), followed by Streptocycline 500ppm (21.12%). Maximum intensity at first spray of 2 Bromo 2 Nitro propane 1,3diol 250ppm (32.72) was observed. Rest of all the treatments found superior as against unsprayed treatment (33.26%).

There were no control of pathogen in single spray. After the first spray there was increase in per cent disease severity to a range of 19.16 % to 33.26 %.

At the end of second spray significantly lowest disease severity of 15.94 % was recorded by the treatment Streptocycline 500ppm + Copper oxychloride 0.25% but was at par treatment (17.29%) 2Bromo500ppm + copper oxichloride 0.25% (17.29 %) Streptocycline 250ppm + Copper oxychloride 0.25% followed by 2Bromo 250ppm + copper oxychloride 0.25% (19.12 %), all the treatments found superior as against unsprayed control.

The result obtained after third spray indicated that significantly lowest disease severity of 15.92 % was recorded by the treatment streptocycline + Copper oxychloride followed by the treatment 2Bromo 500ppm + copper oxychloride 0.25% (16.30 %), Streptocycline 250ppm + Copper oxychloride 0.25% (17.60 %), later two chemical found at par in succession Streptocycline 500ppm (18.35%), 2Bromo 250ppm + copper oxychloride 0.25% (18.40%) found superior as against unsprayed control. (38.17%)

The result obtained after fourth spray indicated that significantly lowest disease severity of 12.89% was recorded by the treatment streptocycline + Copper oxychloride followed by the treatment 2 Bromo 500ppm + copper oxichloride 0.25% (14.37 %), 2Bromo 250ppm+copper oxychloride 0.25% (15.96 %), Streptocycline 250 ppm + Copper oxychloride 0.25% (16.16) Streptocycline 500 ppm (17.45%), 500 ppm + copper oxychloride 0.25% (16.30%), Streptocycline 250ppm + Copper oxychloride 0.25 % (17.60 %), later three chemical found at par in succession.

The mean PDI ranged from 15.97 to 36.97. Disease index was reduced significantly in plot treated as compared to untreated control.

Table.9. *In vivo* efficacy of chemicals and agent on per cent disease severity of Xam of Bt cotton

Tr. No	Treatments	Conc.	PDI before spray	Per cent disease intensity (PI)				Mean PDI	PDC
				I	II	III	I V		
T ₁	Streptocycline	250ppm.	15.31 (14.93)	23.36 (13.51)	22.36 (12.92)	21.49 (12.41)	18.83 (10.85)	21.51 (12.42)	41.81
T ₂	Streptocycline	500ppm	14.38 (13.51)	21.12 (12.19)	20.65 (11.91)	18.35 (10.57)	17.45 (10.05)	19.39 (11.18)	47.55
T ₃	2 Bromo 2 Nitro propane 1,3,diol	250ppm	11.17 (15.36)	32.72 (19.09)	27.26 (15.81)	26.64 (15.45)	25.42 (14.72)	28.01 (16.26)	24.23
T ₄	2 Bromo 2 Nitro propane 1,3,diol	500ppm	14.66 (15.06)	30.97 (18.04)	28.25 (16.41)	24.05 (13.91)	23.48 (13.58)	26.68 (15.48)	27.83
T ₅	Streptocycline 250ppm+ Copper oxychloride 0.25%	250ppm+0.25%	19.61 (13.25)	19.42 (11.20)	17.36 (9.99)	17.60 (10.13)	16.16 (9.30)	17.63 (10.15)	52.31
T ₆	Streptocycline 500ppm+ Copper oxychloride 0.25%	500ppm+0.25%	18.12 (13.95)	19.16 (11.04)	15.94 (9.17)	15.92 (9.16)	12.89 (7.40)	15.97 (9.19)	56.80
T ₇	2Bromo250ppm+copperoxychloride 0.25%	250ppm+0.25%	15.70 (15.35)	21.34 (12.32)	19.12 (11.02)	18.40 (10.60)	15.96 (9.18)	18.70 (10.78)	49.41
T ₈	2Bromo500ppm+copperoxychloride 0.25%	500ppm+0.25%	17.56 (15.17)	19.52 (11.25)	17.29 (9.92)	16.30 (9.38)	14.37 (8.26)	16.87 (9.70)	54.36
T ₉	Strepto250ppm.+copperhydroxide0.1 %	250ppm+0.1%	15.34 (12.04)	22.80 (13.18)	22.37 (12.92)	22.03 (12.72)	18.81 (10.84)	21.50 (12.41)	41.84
T ₁₀	Strepto500ppm.+copperhydroxide0.1 %	500ppm+0.1%	20.29 (11.73)	22.48 (12.99)	20.84 (12.03)	19.60 (11.30)	18.59 (10.71)	20.37 (11.75)	44.90
T ₁₁	2 Bromo +copper hydroxide 0.1%	250ppm+0.1%	14.43 (13.98)	28.26 (16.41)	26.37 (15.30)	23.01 (13.30)	21.38 (12.34)	24.75 (14.33)	33.05
T ₁₂	Control		18.53 (15.05)	33.26 (19.27)	37.61 (22.10)	38.17 (22.43)	38.85 (22.86)	36.97 (21.66)	00.00
	SE ±		1.98	1.22	1.12	1.00	0.92		
	CD at 5%		NS	3.60	3.30	2.93	2.69		

*Mean of three replications, Figures in parenthesis are arc sine transformed value

Among the eleven chemicals lowest mean disease severity of 15.97 was recorded by the treatment streptocycline + copper oxychloride and this reduced disease to an extend of 56.80 %. This was followed by the treatment 2Bromo 500ppm + copper oxychloride 0.25% (16.87 %) Streptocycline 250 ppm+ Copper oxychloride 0.25% (17.63 %), 2 Bromo 250 ppm + copper oxichloride 0.25% (18.70) with reduction in disease to an extend of 54.36, 52.31 and 49.41 per cent respectively.

Similar results were obtained by Jagtap *et al.* (2012) worked on management of bacterial blight disease of cotton with chemicals. The results indicated that per cent disease incidence was reduced by copper oxychloride 0.25% + streptocycline 100 ppm (19.36%) followed by carbendazim 0.1% + streptocycline 100 ppm (20.87%) and copper oxychloride 0.25% + agrimycin 100 ppm (22.47%).

Ingole *et al.* (2015) worked on chemical management of bacterial blight of cotton. The result revealed that the minimum percent disease intensity (11.30%) was recorded in treatment of copper oxychloride (0.3%) + streptocycline (100 ppm) of maximum 5 sprays were effective in reduction of 50.80 percent disease.

4.9. Incremental cost: benefit ratio (ICBR) during *Kharif*, 2017.

Results (Table 10) obtained during *Kharif*, 2017 on economics / incremental cost: benefit ratio (ICBR) in respect of various spray treatments revealed that all the treatments significantly reduced the coefficient of disease index (CODEX) and thereby increased the cotton yield and thereby gave significantly maximum gross, additional income and net profit with better ICBR over unsprayed control of the treatments, antibiotics and fungicides were found most effective in reducing the CODEX, increasing the cotton yield , gross income, additional income and net profit with maximum ICBR over unsprayed control. However, Streptocycline500ppm was found most effective which recorded highest gross income (Rs. 478175), additional income (Rs. 338675) and highest net profit (Rs. 104356) with highest ICBR (2.49) Based on

ICBR, the second and third best treatments found were fungicide 2Bromo 500ppm + copper oxychloride 0.25% and strepto 250ppm. + copper hydroxide 0.1% which recorded gross income of Rs. 97245 and 95802.

Thus on the basis of ICBR, the treatments found effective and most economic in the order of merit were Streptocycline 500ppm, 2 Bromo 500 ppm + copper oxychloride, strepto 250 ppm + copper hydroxide 0.1%

Table No 10. Economics of antibiotics and fungicides sprayings for the management of bacterial blight of cotton Cv. Jaadoo BGII during *Kharif*, 2017

	Treatment	Mean PI	Mean PDI	CODEX	Gross monetary returns (Rs ha ⁻¹)	Cost of Cultivation (Rs ha ⁻¹)	Net monetary returns (Rs ha ⁻¹)	B : C ratio
T ₁	Streptocycline250ppm	27.91 (16.21)	21.51 (12.42)	6.00	82627	36812	45812	2.24
T ₂	Streptocycline500ppm	26.40 (15.60)	19.39 (11.18)	5.11	104356	41911	62445	2.49
T ₃	2 Bromo 2 Nitro propane 1,3,diol250ppm	32.43 (18.92)	28.01 (16.26)	9.08	81482	37931	43551	2.15
T ₄	2 Bromo 2 Nitro propane 1,3,diol500pp	29.10 (16.92)	26.68 (15.48)	7.76	91429	42352	49077	2.16
T ₅	Streptocycline 250ppm+ Copper oxychloride0.25%	26.29 (15.24)	17.63 (10.15)	4.63	92116	40924	51192	2.25
T ₆	Streptocycline 500ppm+ Copper oxychloride 0.25%	24.02 (13.90)	15.97 (9.19)	3.83	85058	37114	47944	2.29
T ₇	2Bromo250ppm+copperoxychloride 0.25%	26.63 (15.44)	18.70 (10.78)	4.97	82627	37005	45626	2.23
T ₈	2Bromo500ppm+copperoxychloride 0.25%	25.84 (14.98)	16.87 (9.70)	4.43	97245	40338	56907	2.41
T ₉	Strepto250ppm.+copperhydroxide0.1%	28.49 (16.56)	21.50 (12.41)	6.12	95801	40421	55380	2.37
T ₁₀	Strepto500ppm.+copperhydroxide0.1%	27.55 (16.00)	20.37 (11.75)	5.16	87957	37978	49979	2.32
T ₁₁	2 Bromo +copper hydroxide 0.1%	28.95 (16.86)	24.75 (14.33)	7.16	80034	39533	40501	2.02
T ₁₂	Control	49.11 (26.89)	36.97 (21.66)	18.15	63652	33997	29655	1.87
	SE(m) ±	49.11 (26.89)	36.97 (21.66)		2920	-	1795.00	-
	CD at 5%				4676	-	5332	-

4.10 Host range of *Xanthomonas axonopodis* Pv. *malvacearum*

Host range of *Xanthomonas axonopodis* pv. *malvacearum* consisting of field crops, plantation crops and tree hosts commonly observed near cotton field is presented in table 11 and table 12. Result explicitly indicated that *Xanthomonas axonopodis* pv. *malvacearum* could not infect 10 field crops and, 8 plantation and tree host on artificial inoculation suggesting that these are non hosts of the organisms.

Wernham (1948) found *X. campestris* pv. *malvacearum* was host specific to cotton and did not infect *Brassica oleracea* var. *capitata*, *Cucurbita maxima*, *Hedera helix*, *Papver rhoeas*, *Phaseolus vulgaris*, *Glycine max*, *Prunus persica*, *Saccharum officinarum*, *Hordeum vulgare*, *Triticum aestivum* and *Lycopersicon esculentum*. *Thurberia thespesiodies*, *Eriodendron anfructuosum*, *Jatropha curcas*, *Lochnera pusilla*, *Thespesia populnea*, *Thespesia lambas* and *Phaseolus vulgaris* are reported to be collateral hosts of *X. campestris* pv. *malvacearum* (Verma, 1986; Srinivasan 1994; Singh 1998). *X. campestris* pv. *malvacearum* was also isolated from *Cieba pentandra* (Verma, 1986). The results are in accordance with the findings of Verma (1986) who observed needle prick inoculation of *Albutilon indicum*, *Aristolochia bracteata*, *Hibiscus rosa sinensis*.

Valerie *et al.* (1988) evaluated that *Xanthomonas campestris* pv. *translucens* is the causal agent of bacterial leaf streak of cereal grains and grasses, and individual strains within the pathovar differ in their host range among the cereals. Co-inoculation of a wide-host-range and a narrow-host-range strain resulted in the wide-host-range reaction. Transposon and chemical mutagenesis of the wide-host-range strain *X. campestris* pv. *translucence* 4, pathogenic on Barley, Wheat, Rye, and Triticale, resulted in variants with reduced host range. When pathogenicity was inactivated independently for Barley, Wheat, Triticale, and Rye, wild-type symptoms were retained on the other members in the host range.

Gholve *et al.* (2005) reported the suitability of 11 field crops, 10 plantation crop and tree and 42 weeds as a host to *X. axonopodis* pv.

malvacearum. The field crops plantation crops and tree were not infected by the pathogen. Among the weed *Euphorbia geniculate*, *Acalypha indica*, *Corchorus acutangulus*, *Phaseolus* spp. and *Cassia tora* developed diseased symptoms and thus, were considered as host of *X. axonopodis* pv. *malvacearum*.

Table 11. Effect of inoculation of *Xanthomonas axonopodis* pv. *malvacearum* on field crops.

Sr. No.	Name of the crop	Infection of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>
	Field crops	
1	Soybean (<i>Glycine max</i>)	-
2	Pigeon pea (<i>Cajanus cajan</i>)	-
3	Green gram (<i>Vigna radiata</i>)	-
4	Blak gram (<i>Vigna sinesis</i>)	-
5	Castor (<i>Ricinis communis</i>)	-
6	Maize (<i>Zea mays</i>)	-
7	Wheat (<i>Triticum aestivum</i>)	-
8	Safflower (<i>Carthamus tinctorius</i>)	-
9	Gram (<i>Cicer arietinum</i>)	-
10	Sorghum (<i>Sorghum vulgare</i>)	-

No infection and no colonies of the bacterium on reisolation.

Table 12. Effect of inoculation of *Xanthomonas axonopodis* pv. *malvacearum* plantation crops and tree .

Sr. No.	Name of the crop	Infection of <i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>
	Plantation crops and tree hosts	
1	Mango (<i>Mangifera indica</i> L.)	-
2	Tamarind (<i>Tamarindus indica</i> L.)	-
3	Pomegranate (<i>Punica granatum</i> L.)	-
4	Gauva (<i>Psidium guajava</i>)	-
5	Neem (<i>Azadorachta indica</i>)	-
6	Pimpal (<i>Ficus religiosa</i> L.)	-
7	Babul (<i>Acasia arabica</i>)	-
8	Ashoka (<i>Sarica indica</i>)	-

No infection and no colonies of the bacterium on reisolation.

A decorative green vine with leaves and red flowers is positioned on the left side of the page, extending from the top to the bottom. The vine is thin and elegant, with several leaves and a cluster of red flowers at the bottom. The flowers have white centers and are surrounded by green leaves.

Summary and Conclusions

A decorative green vine with leaves and red flowers is positioned on the right side of the page, extending from the bottom to the middle. The vine is thin and elegant, with several leaves and a cluster of red flowers at the bottom. The flowers have white centers and are surrounded by green leaves.

CHAPTER V

SUMMARY AND CONCLUSIONS

Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* is one of the most important diseases of cotton causing considerable loss. Bacterial blight is a serious threat to successful cultivation of cotton wherever the crop is grown. Keeping in view, the economic importance of the crop and the disease, present investigations were carried out during 2017 and the results obtained are summarized herein.

A roving survey was carried out during Sept-Oct 2017 at different locations in the districts of Nanded and Parbhani for recording the percent diseases severity and incidence. It was recorded 53.80% of bacterial blight of cotton in field. The plant showing typical symptom of bacterial blight of cotton disease was confirm using ooze test. The data presented are of total Twenty six cotton field were recorded in Parbhani and Nanded district of Marathwada region.

The cotton leaves showing typical bacterial blight symptoms were collected from Cotton Research Station, Nanded, VNMKV, Parbhani and used for isolation. The causal organism was isolated from the infected leaves by following the tissue isolation technique using Nutrient Agar medium and Yeast Glucose Chalk Agar medium. Culture of the isolate was purified by streaking single colony on Nutrient Agar medium and Yeast Glucose Chalk Agar medium and was maintained on the Yeast Glucose Chalk Agar slants.

Pathogenicity of the pathogen was proved by Koch's postulates on cotton seedlings cv. Jaadoo BG II in pot culture. Reisolation was done from inoculated plants, which yielded pure culture identical to original culture and was identified as *Xanthomonas axonopodis* pv. *malvacearum*.

The bacterium was an aerobic, motile, Gram-negative rod, occurring singly, with a single polar flagellum. Colony characters of pathogen were also differed in respect of media as circular to irregular, convex to flattened, light yellow to yellow coloured colonies on NA, YGCA

Symptoms of disease included water soaked minute lesions scattered on the under surface of the young leaves. Lesions became brown and then black, and formed angular dead areas with reddish border bounded by veinlets. The lesions extended along the edges of leaves were also forming vein blight. Symptoms Elongated, grayish to sooty black lesions observed on petioles and stems. Moreover small, round, water soaked lesions were observed on the bolls.

The per cent incidence and per cent disease severity was more in Bt cotton cultivars ranging from 24.02 to 32.43% PI and 15.97 to 28.01% PDI, respectively. Among the various chemicals tested against *Xanthomonas axonopodis* pv. *malvacearum*, the treatment streptocycline (500 ppm) + copper oxychloride (0.25%) produced significantly highest inhibition zone of 21.75 mm followed by streptocycline (500 ppm) + Copper hydroxide (0.1%) 20.46 mm.

Chemical management study conducted under field condition indicated significantly low disease incidence and low disease severity in streptocycline (500 ppm) + copper oxychloride (0.25%) which was to the tune of 24.02 per cent (PI) and 15.97 per cent (PDI), respectively as against unsprayed control 49.11 per cent and 36.97 per cent PDI, respectively. The fungicide 2Bromo 250 ppm+ copperoxychloride 0.25% and streptocycline (250 ppm) + copper oxychloride (0.25 %) recorded second and third best treatment, respectively in controlling the bacterial blight disease.

Thus on the basis of ICBR, the treatments found effective and most economic in the order of merit were Streptocycline 500ppm, 2Bromo 500ppm + copper oxychloride, Streptocycline 250ppm + copper hydroxide 0.1%.

Host range studies conducted on *Xanthomonas axonopodis* pv. *malvacearum*, indicated the pathogen could not infect field crops, plantation crops and tree hosts tested as collateral hosts .

CONCLUSIONS

- Thus, from the results obtained on various aspects during present investigations on bacterial blight of cotton (*Xanthomonas axonopodis* pv. *malvacearum*) disease, following conclusions are drawn:
- The pathogen *Xanthomonas axonopodis* pv. *malvacearum* can grow best on Yeast glucose chalk agar medium. Typical colonies of the test bacterium developed were: circular to irregular, convex to flattened, light yellow to yellow coloured.
- The morphological characteristics of the bacterium showed that it was motile, Gram-negative rod, occurring singly, with a single polar flagellum.
- The per cent incidence and per cent disease severity of Bt cotton cultivars was more when compared to the per cent incidence and per cent disease severity.
- All the test chemicals evaluated *in vitro* found that chemical streptocycline, streptocycline + copper oxychloride, 2 Bromo 2 Nitro propane 1,3,diol and streptocycline + copper hydroxide, 2Bromo 250ppm + copper oxychloride 0.25% were most effective against *Xanthomonas axonopodis* pv. *malvacearum* on Bt cotton
- All the test chemicals evaluated *in vivo* found that streptocycline, streptocycline + copper oxychloride, 2 Bromo 2 Nitro propane 1,3,diol and streptocycline + copper hydroxide , 2Bromo 250ppm + copper oxichloride 0.25% were most effective against *Xanthomonas axonopodis* pv. *malvacearum* on Bt cotton.
- The studies of field evalution of antibiotics and fungicide against bacterial blight disease indicated that the chemical viz., streptocycline 500 ppm was most effective and economical for the management of bacterial blight disease with significantly highest cotton and better ICBR.
- All host tested against *Xanthomonas axonopodis* pv. *malvacearum*, for host range studies found that it could not infect field crops, plantation crops and tree hosts tested as collateral hosts.

*Literature
Cited*

LITERATURE CITED

- Abdo-Hasan, M. H., .Khalil, B., Dedis and MirAli, N. (2008). Molecular characterization of Syrian races of *Xanthomonas axonopodis* pv. *malvacearum*. *J.Pl. Path.*, **90**(3): 431-439.
- Akhtar, M. A. and Khan I.U. (1988). Bacterial blight of cotton and its control. *Pakistan J. of Agri. Res.* **9**(2):202-204.
- Ali Razaghi, Nader Hasanzadeh and Abolghasem Ghasemi (2012). Characterization of *Xanthomonas citri* sub sp. *malvacearum* strains in Iran. *African j. of Microbiol. Res.* **6**(6):1165-1170.
- Allen and Bobby Golden (2012). Response of Cotton Varieties to inoculation with *Xanthomonas axonopodis* pv. *malvacearum* in Mississippi. Mississippi State University.
- Allen and Bobby Golden (2014). Scouting of cotton for bacterial blight. Mississippi State University.
- Ambadkar C. V., Dhawan, A. S. and Shinde, V. N. (2015). Integrated Management of Bacterial Blight Disease (Oily Spot) of Pomegranate Caused by *Xanthomonas axonopodis* pv. *punicae*. **10**(1): 19-23.
- Anonymous (2014). Series of crop biology documents. Biology of cotton. Ministry of Science and Technology, Government of India.
- Antre, S. H., Gadhe, S. K., Abhang, P. B., and Autade R. H. (2016). *In vitro* efficacy of different chemicals, botanicals and bioagents against *Xanthomonas axonopodis* pv. *punicae*. *Int. J. Pure App. Biosci.* **4**(3):112-118.
- Ashish., Arora, A., Gill, P. P. S. and S. K. Jawandha (2016). Effect of agrochemicals on severity of bacterial blight and fruit quality in pomegranate. *Int. J. Agric. Env. and Biotech.* **9**(6):1061-1067.

- Astha Singh, S.S.L. Srivastava and Akram, M. (2007). Studies on bacterial leaf blight of Cotton (*Gossypium* spp). *Int. Journal of Sustainable Crop Production*. **2**(3):25-29.
- Atkinson, G. F. (1891). Black rust of cotton– a preliminary note. *Bot. Gaz. Chicago*.**16**: 61-65.
- Ballard, E. and D. Norris (1923). Bacterial infections of cotton bolls. *Indian J. Agric*.**18**: 40-49.
- Bayles, M. B. and Verhalen L. M. (2005). Recovery of recurrent parent traits when back crossing in cotton. *Crop Sci*. **45**: 2087-2095.
- Bhattiprolu, S.L. (2013). Estimation of crop losses due to bacterial blight disease of cotton. *J. Cotton Res. Dev.***27**(1): 115-118.
- Bhattiprolu, S.L. (2015). Efficacy of kresoxin methyl against foliar diseases of cotton. *J. Cotton Res. Dev.*,**29**(1):112-115
- Bobosha, K. (2003). Characterization of *Xanthomonas campestris* pv. *musacearum* isolates: Casual agent of Enset bacterial wilt disease. M.Sc. Thesis, Addis Ababa University, Ethiopia. 100pp.
- Bora, L. C. and Kataki L. (2014). *Xanthomonas axonopodis* pv. *punicae*-A new threat to pomegranate plants in Assam. *Indian J. Hill farming*. **27**(1):100-101.
- Bradbury, J.F. (1984). *Xanthomonas* Dowsan. (1939). In Bergey's manual of systematic Bacteriology. (Krieg, N.R. and Holt, J.G. eds). Williams and Wilkins, Baltimore., 199-210.
- Carrillo Fasio, J.A., L.Sanchez- Bautista, R.S.Gracia - Estrada, R. Allende – Molar and I. Marquez- Zequera (2001). Races of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye. Present in the

state of Sinaloa, Mexico. *Revista Mexicana de Fitopatologia.*, 19(2): 248-250.

Chand, R. and R. Kishun (1993). Systematic movement of *Xanthomonas campestris* pv. *punicae* (Hingorani and Singh) Dye from leaf to node in pomegranate. *Int. J. Tropical Pl. Disc.* **11** (1): 85-90.

Chauvan, M. S., Kairon, M. S. and Karwas S. S. (1983). Determination of minimum number of effective sprays of agrimycin plus blitox for control the bacterial blight of cotton under Haryana conditions. *Indian J. Mycol. And Pl. Path.* **13**(2):187-191.

Chidambaram, P. (2007). Integrated disease management to reduce yield losses in quality cotton. *Central Institute for Cotton Research.*

CleciDezordi, Antonio Carlos Maringoni, Jose Otavio Machado Menten and Ranata Cassia Camara (2009). Semi-Selective cultural medium for *Xanthomonas axonopodis* pv. *malvacearum* detection in cotton seeds (*Gossypium hirsutum* L.). *Asian Journal of Plant Pathology.* **3**(2): 39-49.

Das, I.K. and J.P. Verma (2003). Plasmids in bacterial associated with bacterial leaf blight of cotton. *Indian Phytopath.* **56**(2): 180-182.

David H., Gent and Howard F. and Schwartz (2005). Management of *Xanthomonas* leaf blight of onion with a plant activator, biological control agents and copper bactericides. *American Phytopathological Society.* **89**(6):631-639.

Dhutraj, D.N. and D.N. Gokhale (2007). Investigation of morphological, cultural, physiological characteristics and staining reaction of seedborne *Xanthomonas* sp. of mungbean. *J.Pl.Dis.Sci.*, 2(1); 37-39.

- Dreo, T., Demsar T. and Ravnikar, M. (2003). Quarantine of *Xanthomonas axonopodis* pv. *phaseoli* in beans. Lectures and papers presented at the 6th Slovenian Conference on Plant Protection, Zrece, 123-127.
- Dyaneshwaram, S., Mahadevan, A. and Rangaswami, G. (1966). A note on comparative studies on twenty eight species of *Xanthomonas*. *Indian Phytopath.* **19**:396-397.
- Ehetisham-ul-Haq, M., Khan, M. A., Javed, T. M., Atiq M. and Rashid, R. (2015). Management of newly emerging bacterial seed and boll rot of cotton with antibiotics, homeopathic products and plant extracts. *Trop. Agric. (Trinidad)*. **92**(3): 196-206.
- El-Sadek, S.M.A., T.I. Abdel-Gawad and N.A. Abdel-Azin (2001). Occurrence of bacterial leaf spot disease on greenhouse- grown pepper in El-Minia, Egypt. *Assiut J. Agric. Sci.*, 32(5):57-69.
- Gena, M., Shah, R. and Mali, B. L. (2008). Host range and efficacy of agrochemicals botanicals and *Bacilli* against cowpea blight bacterium *Xanthomonas axonopodis* pv. *vignicola*. *J. Pl. Dis Sci*, 3(2):144-150.
- Gholve, V. M. and Kurundakar, B.P. (2005) Some new Host of *X. axonopodis* pv. *malvacearum*. *Int. J. of agric.Sci.* **1**: 53-55.
- Goto, M. (1992). Citrus canker. In: Plant diseases international importance. Vol III (J. Kumar, H. S. Chanbe, V. S. Singh and A. N. Mukhopodhyay, Eds.) Prentice - Hall, Englewood Cliff, NJ. PP.170-208.
- Govindappa, Hosagoudar and S. N. Chattannavar (2008). Chemical and biological control of foliar diseases of cotton. *J. Cotton Res. Dev.* **22**(2): 225-228.

- Govindappa, Hosagoudar, S. N. Chattannavar and Srikant Kulkarni (2008). Survey for foliar diseases of Bt cotton. *Karnataka J. Agric. Sci.* **21**(1):139-140.
- Hillocks, R. J. (1992). Fungal diseases of the leaf In Cotton Diseases, *CAB Internl.* 2: 191-238.
- Hussain, T. and Tahir, M. (1993). Chemical control of bacterial blight of cotton. *Pakistan J. of Phytopathol.* **5**(1):119-121.
- Ingole, O. V., Patil, B. R. and Gade, R. M. (2011). Chemical and biological management of bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum*. *J. Cotton Res. Dev.* **25**(1): 88-90.
- Ingole, O. V., Unhale, M. A., Nemade, P. W. and Patil, B. R. (2015). Estimation of avoidable seed cotton losses caused by bacterial blight and chemical management of cotton. *J. Cotton Res Dev.* **29**(1): 177-180.
- Islam, M. Z., Khalequzzaman, K. M., Rahman, G. M. M., Islam, M.T. and Hossain, M. M. (2003). Effect of chemicals in controlling bacterial blight of cotton. *Asian J. of pl. Sci.* **2**(7): 539-543.
- Jagtap, G.P., Jangam, A. M. and Dey, U. (2012). Management of bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum*. *Scientific J. of Microbiol.* **1**(1):10-18.
- Jyoti, S. V., Krishnappa, M. and Reddy, K. M. (2005). Detection of *X. axonopodis* pv. *punicae* on pomegranate (*Punica granatum* L.) in Karnataka. *J. of Mycol. and Pl. Path.* **35**(3): 518-519.
- Kaleem Ullah Drishak, Syed Atif Hasan Naqvi, Rashida Perveen and Usman Atif (2014). Studies on isolation, identification and assessment of bacterial blight of cotton through some dispersal mechanism of *Xanthomonas axonopodis* pv. *malvacearum*. *Int. J. of Biol. Sci.* **1**(6):36-45.

- Kavitha, R. and S.Umesha (2007). Prevalence of bacterial spot in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. *Crop Protec.*, 26(7): 991-997.
- Khabbaz Sala Eddin, Veeramuthu Valluvaparidasan Duraisamy Ladhalakshmi and Rethinasamy Velazhahan (2010). Management of bacterial blight of cotton using a mixture of *Pseudomonas fluorescens* and *Bacillus subtilis*. *Plant Protect. Sci.*, 46(2): 41-50.
- Khan, M.A. (1995). *In vitro* sensitivity of *X. campestris* pv. *malvacearum* to agrimycin-100 and its effect on cotton plant in relation to symptom expression. *Pakistan J. Phytopathol.* 7 (2): 199-201.
- Kirkpatrick, Cliff Coker, Tom Barber and Craig Rothrock (2011). Bacterial blight of cotton found in Arkansas. Southeast Research and Extension Center.
- Koenning, S. (2004). Bacterial blight (Angular Leaf Spot) of cotton. Cotton Disease Information Note No.3.
- Krishna, A. and Nema, A. G. (1983). Evaluation of chemicals for the control of citrus canker. *Indian Phytopath.* **36**: 348-350.
- Kumar, R., Shamarao, J. M., Yenjerappa, S. T. and H. B. Patil (2009). Epidemiology and management of bacterial blight of pomegranate. Caused by *Xanthomonas axonopodis* pv. *punicae*. *Acta. Hort.* 8(18): 291-296.
- Kumar, R., Shamarao, M. R., Jahagirdar, S. T., Yenjerappa and Patil, H. B. (2006). Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. *ISHS Acta Horticulture*.
- Lokesh, R., Erayya, K.M.,Kumaranag, N., Chandrashekarand Khan, A.N.A. (2014). *In vivo* efficacy of some antibiotics against bacterial blight

- of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. *Int. Res. J. of Biol. Sci.***3**(1): 31-35.
- Malavolta, V.A.Jr., I.M.G. Almeida, M.H. Sugimosi and I.J.A.Ribeiro (1999). Occurrence of *Xanthomonas campestris* pv. *viticola* in grape in Brazil. *Summa. Phytopathol.*, 25(3): 262-264.
- Manjula, C. P., Khan, A.N.A. and Ravikumar, M. R. (2003). Management of bacterial blight of Pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae*. *Indian Phytopath.***56** (3):342.
- Manjula, C.P., Khan, A.N.A. and Ravikumar, M. R. (2003). Management of bacterial blight of Pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae*. *Indian Phytopath.***56**(3): 342.
- McGuire, R.G., J.B.Jones and M. Sasser (1986). Tween media for semi-selective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. *Pl.Dis.*,70:887-891.
- Mehrotra, R.S.(1988). Seed borne disease. Plant Pathology, Edn.1, Tata McGraw Hill Publishing Company Ltd. pp. 606-615.
- Meshram, M.K. and Sheo-Raj (1988a). Seed cotton yield and fibre quality as influenced by different grades of bacterial blight under rainfed conditions. *Indian J. Plant Protection*, 16(2): 257-260.
- Meshram, M.K. and Sheo-Raj (1992). Effect of bacterial blight infection at different stages of crop growth on intensity and seed cotton yield under rainfed conditions. *Indian J. Plant Protection*, 20(1): 54-57.
- Meshram, M.K. and Sheo-Raj (1988b). Assessing yield losses due to bacterial blight in cotton. National symposium on Phytobacteriology, Madras, 105-109.

- Mishra, A. K. and Om Prakash (1992). Bacterial canker of mango, Incidence and control. *Indian Phytopath.* **45** (2): 172-175.
- Mishra, S.P. and Krishna, A.(2001). Assessment of yield losses due to bacterial blight in cotton. *J. Mycol. Pl. Pathol.*, 31(2): 232-233.
- Mogle, T. R., Surywanshi, A. P., Badgire, D. R., Dadke, M. S., Magar, S. J. and Harde, A. L. (2009). *In vitro* efficacy of botanicals and bioagents against *Xanthomonas axonopodis* pv. *punicae*. National Conference on frontiers in Plant Diseases, Diagnosis and Management, held at Maharashtra Mahavidyalay, Nilanga.on Jan. 3(4):28.
- Mondal, K. K., Rajendran, T. P., Phaneendra, C., Mani, C., Sharma, J., Sharma, R., Pooja., Verma, G., Kumar, R., Singh, D., Kumar, A., Saxena, A. K. and Jain, R. K. (2012). The reliable and rapid polymerase chain reaction (PCR) diagnosis for *Xanthomonas axonopodis* pv. *punicae* in pomegranate. *African J. Microbiol. Res.* **6**(30): 5950-5956.
- Muhammad Imran Hamid, Muhammad Aslam Khan, Zafar Iqbal, Muhammad Usman Ghazanfar, YasirIftikhar and Naeem Akhtar (2012). Correlation of environmental conditions with bacterial blight disease of cotton (*Gossypium hirsutum* L.)*Pak. J. Phytopathol.* **24**(1): 39-43.
- Muhammad, S., Rashid, A., Muhammad E., Muhammad T. J., Jamil, H. Muhammad Mudassir, Muhammad, F., Muhammad, L., Munir, A., Chohan, Masood, A. and Kamran, A. (2013). *In vitro* evaluation of chemicals and plant extracts against colony growth of *Xanthomonas axonopodis* pv. *malvacearum* causing bacterial blight of cotton. *European J. of Exptl. Biology.* **3**(1):617-621.
- Mukesh Gena, Rakesh Shah and Mali, B. L. (2008). Host range and efficacy of agrochemicals botanicals and *Bacilli* against cowpea blight bacterium *Xanthomonas axonopodis* pv. *vignicola*. *J. Pl. Dis. Sci*, **3**(2):144-150.

- Nath, J., Gurha, S. N. and N.K.Taneja (1981). Sensitivity of *Xanthomonas malvacearum* the incitant of bacterial blight of cotton to chemicals. *Indian J. Mycol. Pl. Pathol.* **11**(2): 255-257.
- Ogunjobi, A.A., O.E. Fagade and G.O.Dixon (2010). Physiological studies on *Xanthomonas axonopodis* pv. *manihotis* (Xam) strains isolated in Nigeria. *European Journal of Biological Sciences*, **2**(4): 84-90.
- Opara, E.U. and F.J.C. Odibo (2009). Studies and Characterization of Bacterial Spot Pathogen of Tomato *Xanthomonas campestris* pv. *vesicatoria*. *Journal of Molecular Genetics*, **1**(2-4): 35-43.
- Patel, M. K. and Kulkarni (1948). *Curr. Sci.* **17**: 243. (Cited from Srinivasan, K.V., 1994).
- Pathak, A.K. and Shailesh Godika (2006). Management of bacterial blight of cotton *Xanthomonas axonopodis* pv. *malvacearum* through chemicals. *J. of Mycol. and Pl. Path.* **36**(1): 35.
- Patil, A. V. and Karle, A. R. (1990). Pomegranate In: Fruits, tropical and subtropical. *J. Cotton Res. Dev.* **11**(2): 206-208.
- Patil, M.R. and Ghoderao, B. N. and C.U.Patil. (1997). Chemical control of bacterial blight including green boll rot phase of cotton.
- Patil, P.V., Patel, J. R. and Patel, U. G. (2003). Assessment of avoidable yield losses caused by bacterial blight in cotton. *J. Cotton Res. Dev.* **17**(1): 45-47.
- Patole, S.P., Sulunkhe, R.S and Phapale, A.D. (2016). Survey and occurrence of Bacterial blight of cotton. *Int. J. of pl. protection.* **9**(10): 527-531.
- Peterson, Y., Mansvelt, E. L., Venter, E. and Langenhoven W. E. (2007). Detection of *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight of pomegranate. *Australian. J. of Pl. Path.* **39**: 544-546

- Prashant B., Sandipan, G.R. Bhandari, R. D. Patel and Solanki, B. G. (2017) Survey and Status of Different Diseases of Cotton under South Gujarat Region. *Indian Int. J. of Current Microbiol. and Appl. Sci.* ISSN: 6(9):1362-1367
- Raghuwanshi, K. S., Hujare, B. A., Chimote, V. P. And Borkar, S. G. (2013). Characterization of *Xanthomonas axonopodis* pv. *punicae* isolates from western Maharashtra and their sensitivity to chemical treatments. *Int. J. Life Sci.* 8(3): 845-850.
- Rampandu, S., Sitaramaiah, K., Subbarao, K. and Prasadaraao, M. P. (1979). Screening of cotton germplasm against bacterial blight caused by *X. campestris* pv. *malvacearum*. *Indian Phytopath.* 32: 486-487.
- Rathore. B.S. (2010). Efficacy of streptocycline and plant extracts against bacterial leaf spot disease caused by *Xanthomonas axonopodis* pv. *Vigna radiatae* of greengram. *Indian Phytopath.* 63 (4):384-386.
- Raut.S.A., Ingole, O. V., Raut, B. T. and Bhoje, B B. (2010). Evaluation of bioagents, botanicals and chemicals against bacteria blight of cotton. *J. Pl. Dis. Sci.* 5(1):83-85.
- Saeedi M. A., Marefat, A., Behboudi and, K. and Ghasemi, A. (2010). Phenotypic and genetic characteristics of *Xanthomonas citri* sub sp. *malvacearum*, causal agent of cotton blight and identification of races in Iran. *Australasian Plant Pathology.* 39: 440–445.
- Sain, S.K., and Gour, H. N. (2013). Pathological and Physio-biochemical characterization of *Xanthomonas axonopodis* pv. *glycines*, incident of glycine max leaf pustules. *Indian Phytopath.* 66(1):20-27.
- Salha E. K. (2017). Comparative Studies Between *Xanthomonas citri* sub sp. *malvacearum* Isolates. *World J. of Agric. Res.* 5(2): 64-72.

- Schaad, N.W. (1988). Laboratory guide for identification of plant pathogenic bacteria APS press ST Poul Minnosota.
- Schiegel, H.G. (1995). *General Microbiology*, 7th ed. Cambridge University Press.
- Shah, R., Bhatnagar, M. K., Mali, B. L. and Dashora, P. K. (1991). Control of bacterial blight of cowpea. *Indian J. Mycol. Plant Pathology*, 21(3): 251-254.
- Sharma, K.K., Jadhav, V. T. and Sharma, J. (2009). Present status of pomegranate bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae* and its management. *ISHS Acta Horticulture*.
- Sharma, R. R., Thind, B. S. and Singh. N. (1979). *In vitro* and *In vivo* evaluation of chemicals against *Xanthomonas vesicatoria*, the causal agent of bacterial leaf spot of chillies. *Indian J. Mycol. and Pl. Path.* **11**(2):178-182.
- Sheo-Raj and J.P. Verma (1989). Diseases of cotton in India and their management. *Review Tropical Plant Pathology*. 5: 207-254.
- Sheo-Raj, S. (1988). Grading system for cotton diseases. CICR Tech. Bull.7.
- Simpson, D.M. (1956). *Plant Dis. Report*, 40: 549-555. (Cited from Srinivasan, K.V., 1994).
- Singh, H. and Singh Nirmaljit (1988). Evaluation of some chemicals for the control of bacterial blight of cotton Pesticides. **22**(10):13-14.
- Srinivasan, K.V. (1994). Bacterial blight. In cotton diseases, pp. 278-287.
- Sujatha, B. and D.V.R. Sai Gopal (2010). Isolation and characterization of *Xanthomonas axonopodis* from *Citrus aurantifolia christm* (swingle). *The Bioscan*. 5(3): 373-376.

- Supriadi, E.M., Adhi, N., Hasanam, D., Febriyanti and Rahyayuningsih, R. (1996). *Indonesian J. Crop-Sci.* **11**(2): 31-39.
- Suryawanshi, A. P., Mogle, T. R., Somwanshi, S. D., Magar, S. J. and B. D. Wadulkar (2009). Efficacy of antibiotics and fungicides against oily spot disease of pomegranate. National Conference on Frontiers in Plant Disease, Diagnosis and Management, held at Maharashtra Mahavidyalay, Nilanga. pp:28.
- Swanepoel, A. (1990). Bacterial blight in cotton. Farming in South Africa, (G.7): 1p.
- Thirumalachar, M.J., Patel, M. K., Kulkarni, N. B. K. and Dhand G. W. (1956). Effect of *in vitro* of some antibiotics on thirty two *Xanthomonas* sp. Occurring in India. *Phytopathology*, 46:486.
- Thwipa, M.T., Osman, S. A., Mohammed E. A., Elamin, H. B. Osman, A., Mahdiin, A. (2016). *In vitro* screening of some biocontrol agents against *xanthomonas axonopodis* pv. *malvacearum* isolated from infected cotton plant. *Int. J. of Agric., Forestry and Plantation*. **2**: 2462-175
- Upasana, R. and Verma, K. S. (2001). Pathogenic potential of *Xanthomonas axonopodis* pv. *punicae* and field response of different cultivars. *Pl. Dis. Res.* **16**(2):198-202.
- Uppal, B.N. (1948). Disease of cotton in India. Indian Centre Cotton. Comm., Bombay, India, pp: 32.
- Venkateswarlu, C. H. and Ramapandus (1992). Compatibility of fungicides and bactericides with insecticides in the control of citrus canker and leaf minor in acid lime. *Indian J. Pl. Prot.* **20**:27-31.
- Verma, J.P. (1986). Bacterial blight of cotton. CRC Press, Florida, USA, pp 278.

- Verma, J.P. and R.P.Singh (1974). Control of bacterial blight of cotton. *Pesticides*, 7(1): 16-17.
- Verma, J.P., Singh, R. P. and Nayak, H. L.(1975). Laboratory evaluation of chemical against *X. campestris* pv. *malvacearum* the incidence of bacterial blight of cotton. *Indian Phytopath.* **28**(2): 171-174.
- Vinay, B., Raghavendra, L. S. and Harischandra S. P. (2009). Role of cultivars, physical, chemical and Organic treatments in the management of bacterial blight of cotton. *Archives of Phytopathology and Plant Protection*, **42**(12):1101-1108.
- Vincente. J. G, Conway, J., Roberts, S. J. and Taylor, J. D. (2001). Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. *American Phytopathological Society*. **91** (5) :492-499.
- Wenham,O.C. (1948). The species value of pathogenicity in the genus *Xanthomonas*. *Phytopathology*. 38:283
- Wu, W. C., Chen, N. R. Y., Wu, J. C., Su, L., Wang, C. F. and Uneg, S. H. (1980). *Xanthomonas axonopodis* pv. *mangiferae indicae*, its phages and its sensitivity to chemicals. *Plant Protection Bulletin, Taiwan*, **22**(3):287-296.
- Yenjerappa, S. T. (2009). Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Singh) Ph. D. Thesis, submitted to Univ. of Agri. Sci. Dharwad (India). pp: 79-90.

A decorative green vine with leaves and red flowers is positioned on the left side of the page, extending from the top to the bottom. The vine is thin and elegant, with several leaves and a cluster of red flowers at the bottom. The flowers are bright red with white centers and yellow stamens. The leaves are green and have a serrated edge.

Thesis Abstract

A decorative green vine with leaves and red flowers is positioned on the right side of the page, extending from the bottom to the middle. The vine is thin and elegant, with several leaves and a cluster of red flowers at the bottom. The flowers are bright red with white centers and yellow stamens. The leaves are green and have a serrated edge.

MANAGEMENT OF BACTERIAL BLIGHT (*Xanthomonas axonopodis* pv. *malvacearum*) IN RAINFED Bt COTTON”

Name of student : kharat k.y. **Reg. No.** 2016A /95M
Research Guide : Dr. P.K. Dhoke
Designation : Assistant Prof. (Plant Pathology)
Cotton Research Station, Nanded
VNMKV, Parbhani

ABSTRACT

Bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum* is one of the most destructive diseases of cotton (*Gossypium spp.*), causing severe yield losses upto 40.19 per cent. Present investigations on the disease (*Xam*) were carried out during 2017-18 to fulfill the objectives defined, at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

A roving survey has been carried out during sept-oct 2017 .of different location Nanded and Parbhani for recorded the percent diseases severity and incidence 53.80% of bacterial blight of cotton in field. The plant showing typical symptom of bacterial blight of cotton disease conform through ooze test. The data presented in total Twenty six cotton field were observed in Parbhani and Nanded district of Marathwada region.

The test bacterium was successfully isolated from infected leaves showing typical symptoms of bacterial blight, yielded yellow, smooth, flat glistening, round colonies on NA and YGCA media. Based on the morphological, cultural, and the pathogenicity, the bacterium was identified as *Xanthomonas axonopodis* pv. *malvacearum*.

Symptoms was observed on three different cultivars of Bt cotton were not observed due to unfavourable climatic condition for the development of pathogen. The percent disease incidence was found more in Bt cotton cultivars ranging from

24.02 to 32.43% PI and 15.97 to 28.01% PDI respectively whereas the per cent incidence and per cent disease severity was less

Among the various chemicals tested under *in vitro* condition against *Xanthomonas axonopodis* pv. *malvacearum*, streptocycline streptocycline (500 ppm) + copper oxychloride (0.25%) produced significantly highest inhibition zone of 21.75 mm followed by streptocycline (500 ppm) + Copper hydroxide (0.1%) 20.46 mm.

Under *in vivo* condition, significantly low per cent disease incidence and per cent disease intensity of Bt cotton were recorded in treatment streptocycline (500 ppm) + copper oxychloride (0.25%)) to the tune of 24.02 per cent (PI) and 15.97 per cent (PDI) respectively as against unsprayed control 49.11 per cent and 36.97 per cent respectively. The fungicide 2Bromo 250 ppm+ copperoxychloride 0.25% and streptocycline (250 ppm) + copper oxychloride (0.25 %) recorded second and third best treatment respectively in controlling the bacterial blight disease.

Thus on the basis of ICBR, the treatments found effective and most economic in the order of merit were Streptocycline 500ppm, 2Bromo 500ppm + copper oxychloride, strepto 250ppm + copper hydroxide 0.1%. 2.49, 2.41 and 2.37 respectively.

On *Xanthomonas axonopodis* pv. *malvacearum*, host range studies conducted where found that it could not infected field crops, plantation crops and tree hosts tested as collateral hosts .



Appendix



APPENDIX-I

Composition of different media

1. Nutrient Agar (NA)

Peptone	:	5.0 g
Beef Extract	:	3.0 g
Sodium Chloride	:	5.0 g
Agar	:	15.0 g
Distilled Water	:	1000 ml

2. Yeast Glucose Chalk Agar (YGCA)

Yeast	:	10.0 g
Glucose	:	10.0 g
CaCO ₃	:	20.0 g
Agar	:	20.0 g
Distilled Water	:	1000 ml

3. Nutrient Broth

Peptone	:	5.0 g
Beef Extract	:	3.0 g
Sodium Chloride	:	5.0 g
Distilled Water	:	1000 ml

4. Yeast Glucose Chalk Broth

Yeast	:	10.0 g
Glucose	:	10.0 g
CaCO ₃	:	20.0 g
Distilled Water	:	1000 ml



Vitae

VITAE

PERSONAL DETAILS:-

- 1. Name** : Mr. Kharat Kakarao Yadavrao
2. Date of Birth : 09/04/1994
3. Gender : Male
4. Religion : Hindu- Maratha
5. Present Address : At/post Hasnabad, Tq. Bhokardhan, Dist. Jalana
6. Contact : 9518930998
7. Email ID : kakakharat1111@gmail.com
8. Educational Qualification :

Course	Passed Yr	Board/ University	Institute	Percentage
H.S.C	2012	Aurangabad	Saint Dnyaneshwar Mahavidyalay, Hasanabad	46.36 %
B.Sc. (Agriculture)	2016	VNMKV, Parbhani	College of Agriculture, Kharpudi	7.42%

Place : Parbhani

Date :

Signature

Mr.Kharat K. Y.